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FUNCTIONAL CARDIORESPIRATORY TOXICITY SCREENING OF CANDIDATE ANTIPARASITIC DRUGS AND ANTIDOTES FOR CHEMICAL POISONS

Subtitle: STUDY OF THE EFFECTS OF DRUGS UPON THE CARDIOVASCULAR AND RESPIRATORY SYSTEMS

ANNUAL REPORT

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Summary

During this past year we have:

1. Completed study of Cardiovascular and Pulmonary Effects of Pyridostigmine Bromide in the Dog: Correlation with Blood Cholinesterase Inhibition. A copy of this report (submitted Feb., 1986) is attached (Section I).

2. Written a protocol to study the Effects of Chloroquine and Mefloquine Individually and in Combination Upon Automaticity, Rhythmicity and Dynamics of the Heart. A copy of this protocol (submitted 1 May, 1986) is attached (Section II).

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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Section I

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* 1. Baseline values, 2. Blood Chemistries, 3. Summary of ECG changes, 4. Range of baseline values and range of responses.

SUMMARY

A range of doses of pyridostigmine were infused intravenously into anesthetized beagle dogs to determine the minimal effective and the maximum tolerated doses upon cardiovascular and pulmonary function and blood cholinesterase (Che) activity. Doses of 0.5 and 5.0 mg/kg infused over 15 minutes were found to fulfill these criteria; a dose of 2 mg/kg produced intermediate effects. The 0.5 mg/kg dose of pyridostigmine produced a slight (10%) fall in tidal volume and airways compliance, a 200% increase in airways resistance, but no change in respiratory rate or minute volume. This dose also produced a 10% fall in heart rate, a small increase in cardiac output (8%), stroke volume (20%) and pulmonary artery (15%) and wedge pressure (2 mmHg), and a 35% fall in blood Che activity. The 5 mg/kg dose produced a 30% fall in tidal volume, a 40% fall in airways compliance, a 1000% rise in airways resistance, a 100% rise in respiratory rate, and a 30% rise in minute volume. This dose also produced a rise during infusion and subsequent fall in systolic pressure, a 30% fall in diastolic pressure, a 45% fall in heart rate, a 25% increase in cardiac contractility, an 80% rise in stroke-volume, a rise and fall in cardiac output, an increase in pulmonary vascular resistance (50%) and wedge pressure (9 mmHg), a 15-20 msec increase in P-R interval and a 60% fall in Che activity. This dose also appeared to slow repolarization of the ventricle as Q-T interval was lengthened by 17%. The 2mg/kg dose generally produced effects which were intermediate between those of the high and low doses. Mucous production and defecation were especially notable in the high dose. **Conclusions: Pyridostigmine in doses of 0.5 to 5 mg/kg i.v. in the dog produced a dose-related inhibition of plasma cholinesterase activity. Additional changes were: heart rate was reduced; cardiac contractility (dp/dt) was increased; stroke-volume was raised; cardiac output was thus only variably affected; airways resistance was markedly increased.**

INTRODUCTION

Pyridostigmine bromide is a reversible inhibitor of acetylcholinesterase. This agent increases plasma and tissue half-life and the biological effects of acetylcholine. The main therapeutic use of this agent is for management of myasthenia gravis, a disease manifested by skeletal muscle weakness resulting from reduced density of acetylcholine receptors at the neuromuscular junction.

A recent report has given information about the cardiopulmonary actions of pyridostigmine Br in conscious dogs (Ehrlich et al, 1985). At a dose of 1 mg/kg (i.m.) pyridostigmine increased respiratory rate and minute volume as well as airways resistance, in resting dogs. Heart rate, cardiac output, and blood pressure were not altered. In exercising dogs, respiratory rate increased, but minute volume and airway resistance were unchanged. Heart rate was lowered, but cardiac output was not changed. Pulmonary artery pressure was elevated in both resting and exercising animals. Fifteen minutes after injection (1 mg/kg, i.m.), a plasma cholinesterase level of 40% of control was reached.

Our studies determine and correlate the cardiovascular and pulmonary actions of graded intravenous doses of pyridostigmine Br in the anesthetized dog. The range of doses studied was from that producing 1) a small but definite effect upon cardiovascular and/or pulmonary function, to a dose which produced 2) severe changes in cardiopulmonary function, just short of death. A dose producing effects intermediate to these extremes was also determined. In addition, these studies provide a correlation of blood cholinesterase activity with cardiovascular and pulmonary function before, during and after administration of pyridostigmine. The formulation of this drug which was utilized was Mestinon® sterile solution (5 mg/ml).

Our Preliminary Studies: In range finding studies in 12 anesthetized mongrel dog preparations, we found that a total dose of 5 mg/kg (i.v.) of pyridostigmine (Mestinon®) appeared to be the maximum tolerated. Higher doses of 6 and 7 mg/kg produced death by apparent respiratory paralysis in 2 of 3 dogs tested. A dose of 0.5 mg/kg was the lowest dose which consistently produced notable changes in cardiovascular and pulmonary function. A dose of 2 mg/kg produced intermediate effects.

Initial Studies

In May, 1985, we began experimental work according to the OUTLINE OF STUDIES below. Ten experiments were completed using mongrel dogs before our research was halted in June, 1985, due to a new Department of Army policy which forbade the use of pound dogs in sponsored research. Results of these studies are given in Appendix F. Studies were reinitiated in August, 1985 with pure-bred beagle dogs.

OBJECTIVES

The purpose of these experiments was to determine the effects of varying degrees of inhibition of blood cholinesterase upon cardiorespiratory variables produced by the range of doses of pyridostigmine; the lowest dose at one end, produced only minor cardiopulmonary effects and, the largest dose, at the other end, caused severe changes just short of death. We have attempted to correlate functionally graded changes in cardiovascular and pulmonary performance in response to pyridostigmine.

METHODS

I. Drug Preparation and Delivery

Pyridostigmine Br (mw = 261.14), 1-methyl-3-hydroxypyridinium bromide dimethylcarbamate, was purchased from Roche Laboratories as Mestinon® [Lot #0102 for injection, 5 mg/ml, 2 ml/ampul]. Samples of this lot were analyzed for purity and content by SRI International, Menlo Park, California. The report of this organization revealed that the injectable solution met USP specifications for content (5.00 mg, s=0.05 mg pyridostigmine), pH (5.19) and volume (2.26 ml) per ampule (Petesch et al, 1985). Lot #0101 was used in experiments with mongrel dogs. Analysis of this lot indicates that it also met USP requirements. This drug was diluted daily for each experiment with normal saline to an appropriate concentration to deliver the desired dose in a volume of 29 ml (1.91 ml/min x 15 min). Aliquots of the commercial vial contents were accurately weighed at 21°C to measure our addition of proper amounts of test drug to the infusate.

II. Animals

Beagle dogs of either sex, 9 to 12 months old, weighing between 6.7 and 14.2 kg were purchased from Ridglan Animal Care Systems (Mt. Horeb, WI) for these studies. The dogs were certified to be in excellent health and filaria free and were examined by a University of Tennessee veterinarian before use. Only dogs with normal ECG profiles were used. Food was withheld for twenty-four hours prior to the experiment. Dogs were anesthetized with pentobarbital Na, 30 mg/kg, intravenously and maintained with supplemental injections of 1.0 mg/kg as necessary to maintain stable anesthesia level. Corneal and plantar reflexes, response to pain, and respiratory rate (16-20 breaths/min) were used in "titrating" the dog to the desired level of anesthesia. Body temperature was monitored through the Swan-Ganz catheter and maintained by

heat lamps between 37-38°C. Upon completion of the experiment we euthanized the dog by injecting 10 ml of saturated KCl solution i.v. and monitored to cardiac asystole and respiratory apnea.

III. Cardiovascular Measurements

Cardiovascular variables were measured and/or computed using a Grass Model 7B polygraph and a Buxco Cardiovascular Analyzer and were recorded on both the polygraph and a Texas Instruments Model 675 data terminal.

N.B. See Appendix G for details of instrument calibration procedures.

All catheters for pressure measurements were filled initially with heparinized saline (80 units/ml) and flushed with heparinized saline as needed to maintain patency. A femoral artery was catheterized with polyethylene tubing (PE 260) advanced to the thoracic aorta and connected to a Statham P23AC pressure transducer for measurement of arterial blood pressure. The left carotid artery was cannulated with a Millar® Mikro-tip® catheter pressure transducer (Model PC-350, size 5F) for high fidelity measurement of left ventricular pressure (LVP). The rising slope of the LVP was differentiated to give dP/dt, an estimate of myocardial contractility. The left ventricular pressure signal was also used to record left ventricular end diastolic pressure. Heart rate was recorded via a Grass cardiotachometer triggered by the R wave of the Lead II ECG. Short strips of standard leads were recorded every 10 minutes at 25 mm/sec and Lead II rhythm strip was recorded every 10 minutes.

One femoral vein was catheterized to the level of the heart for drawing venous blood samples (approx. 4 ml) and the other femoral vein was cannulated for drug infusion. Arterial blood samples (approx. 5 ml) were drawn from the

aortic pressure line at selected time points. Samples were taken in glass syringes rinsed with heparinized saline following evacuation of the static volume in the cannulae. Syringes containing blood were immediately capped with tight-fitting rubber nipples and put in ice until analysis. The first samples drawn were designated as -15 minutes. At time 0, just prior to onset of the infusion of drug or vehicle, another set of blood samples were taken. Additional sets of blood samples were taken at +7.5, +15, +25, +45, +85, and +115 minutes. We drew one ml of arterial blood and transferred the blood to a glass test tube rinsed with heparinized saline, and lysed the cells by freezing. The samples were then thawed and used for analysis of cholinesterase activity. (See section V, Blood Analysis). Microhematocrit was assessed using venous blood. Withdrawal of blood was performed by a person other than the operator of the blood gas analyzer.

Cardiac and Pulmonary Vascular Function

For the measurement of cardiac output an Instrumentation Laboratories thermal dilution cardiac output computer (see appendix for theory of operation) was used. Cardiac output, pulmonary artery and wedge pressure were determined from this same catheter. Outputs were obtained in duplicate at given time points by injection of 3 ml of saline at zero degrees C and a subsequent computer integration of the temperature change vs time curve as detected by the thermistor. The two determinations for each point were taken at the same place in the respiratory cycle and averaged. A Swan-Ganz type catheter (Swan et al., 1970) with an extra injection port and a temperature sensor was connected to a Statham P23Gb pressure transducer (ultra low volume displacement) and was inserted into the right jugular vein. The balloon at the catheter tip was inflated and the catheter "floated" through the right

atrium and the right ventricle into the pulmonary artery. The distinctive changes in pressure tracings identify these locations. The balloon tip was advanced into the pulmonary artery until an abrupt drop in pressure and cessation of pulsation indicate that the balloon had occluded a secondary branch of the pulmonary artery. The balloon was then deflated (restoring the pressure pulsations) and the catheter advanced a few more mm, maintaining the normal pulmonary artery pressure pulsations. The catheter was then securely tied in place. Upon re- inflation of the balloon tip (for 15 sec), pulmonary wedge pressure (an indication of left atrial pressure) was recorded at desired time points. With the balloon deflated, pulmonary artery pressure was continuously recorded.

Pulmonary Vascular Resistance (PVR) was calculated according to the formula: PVR = (PAP - LVEDP)/CO, where PAP = pulmonary artery pressure, mm Hg; LVEDP = left ventricular end diastolic pressure, mm Hg; CO = cardiac output or pulmonary artery blood flow, L/min. Interpretation of the drug's action took into account possible flow-induced changes in the pulmonary vasculature.

IV. Pulmonary Ventilatory Measurements

N.B. See Appendix G for instrument calibration procedures and details of pulmonary ventilatory measurements.

We estimated the pulmonary function of the dogs breathing room air unassisted via an endotracheal tube with a side arm that was connected directly to a mesh screen Fleish pneumotachograph. We measured the pressure difference across the pneumotochograph screen with a Validyne® differential pressure transducer, Model MP45-24. The resultant signal, when calibrated against a known air flow, corresponds to the tidal airflow rate and, in turn, when inte-

grated, yields tidal volume. An esophageal tube (Porter ID 6.5) was inserted into the esophagus for the estimation of intrapleural pressure. The pressure difference between the airway and esophagus, or transpulmonary pressure, was measured by a second Validyne® differential pressure transducer, Model MP45-14. Dynamic airway resistance ($\Delta P/\Delta F$) and dynamic airway compliance ($\Delta V/\Delta P$) were computed using a Buxco Electronics Pulmonary Mechanics Computer Model 6. Airway resistance and compliance and tidal volume were recorded as analog signals with a Grass Model 7b polygraph and as digital values with a Texas Instruments Model 765 data terminal.

V. Blood Analyses

All samples were analyzed within 10 minutes for O_2 content by Cheryl Reick using the Corning Model 165/2 Blood Gas Analyzer . Each sample (4 ml each of arterial and venous blood) was analyzed in triplicate. Calibrations were made with standard gas mixtures.

Assay of whole blood cholinesterase was performed as described by Ellman et al. (1961) and Siakotos et al. (1969). We froze the blood samples 7-14 days prior to cholinesterase determinations. Tests in our laboratory have shown that there is no difference in results obtained from fresh blood or blood frozen for two weeks (Appendix H). Briefly, this method for assessing total whole blood cholinesterase activity involves the 30 min incubation of 10ml of blood within 300 µl of a buffer-salt-detergent solution containing [acetyl-^{14}C]-choline Iodide (New England Nuclear 4.4 mCi/mmol) and cold acetylcholine at a concentration of 10^{-3}M ($6\lambda10^{6}$ cpm/ml). The reaction was stopped by addition of a suspension of Amberlite IRP-69 resin in dioxane. This resin binds unhydrolyzed (intact) acetylcholine. The resultant mixture

was centrifuged (900 g for 1 min) and an aliquot of the supernatant solution containing free ^{14}C-acetate was measured by a liquid scintillation counter. Non-enzymatically (non-specifically) produced free ^{14}C-acetate was determined by the regular assay, but by use of reaction mixture containing water rather than blood.

VI. Other Observations

Because this particular drug often causes increased mucous secretion within 5-10 mins of the start of infusion, the volume of mucous produced and released from mouth and nose was estimated by the use of a funnel and graduated cylinder under the dog's snout. Following euthanasia with KCl, the lungs were inspected grossly for abnormalities and a lung lobe was tied off and placed in water for an estimate of density. The heart and lungs were inspected for presence of filaria.

Because pilot studies showed that this drug induces defecation, frequency of defecation was noted.

VII. Data Presentation

All measurements called for in this protocol are presented in tabular form for each experiment using absolute values for each variable. Summary graphs have been constructed to show mean-percentage change from baseline (or control) which are designated 100%. Several variables are plotted as mean absolute values. Mean blood gases and hematocrit are given in tabular form. Variability for each measurement is given as mean ± 1 S.E.M. Standard error of the mean (S.E.M.) is estimated as standard deviation (σ) ÷ $\sqrt{N-1}$(Spence et al. 1968). For purposes of correlation of events, several related variables are plotted on a single graph. In addition, samples of actual tracings are used to illustrate typical ECG responses to various doses. Values were determined for most variables at 10 min intervals. Because spurious and

unrepresentative values may occur for any cardiac beat or respiratory cycle, a series of values for each variable for a timepoint were inspected and the representative value was chosen for that epoch.

OUTLINE OF STUDIES

We used the following protocol and experimental scheme: Approximately 60 minutes were required following induction of anesthesia to perform the necessary surgery, cannulation procedures, and to establish calibrations. This was followed by a stabilization period of 20 to 30 minutes to insure that all variables were stabilized, and this, in turn, was followed by a control period of 15 minutes during which data were recorded. The drug infusion was then begun and continued for 15 minutes. There was a post-infusion period of 120 minutes for observation of recovery.

I. Observations - 15 minute control period

A. Cardiovascular Measures

1. arterial blood pressure - continuous
2. left ventricular pressure - continuous
   a. dP/dt - continuous
   b. left ventricular end diastolic pressure - continuous
3. electrocardiogram - Lead II, strips recorded at 25 and 100 mm/sec for analysis (-15 and 0)
4. heart rate - continuous; by cardiotachometer
5. pulmonary vascular
   a. pulmonary artery pressure - continuous
   b. pulmonary wedge pressure - at 15 minute interval (-15 and 0)
   c. cardiac output - at 15 minute interval (-15 and 0)

d. pulmonary vascular resistance - calculated for the 15 minute interval (-15 and 0)

B. Pulmonary Ventilatory Measures

1. Airways differential pressures - continuous

a. air flow - signal integrated by preprogrammed Buxco computer

b. transpulmonary pressure (bronchial minus esophageal) - signals utilized by preprogrammed computer

2. Airway integrated measures - tidal volume and minute volume - continuous

3. Airways computer measures

a. compliance - continuous = $\Delta V/\Delta P$

b. resistance - continuous = $\Delta P/\Delta F$

4. Respiratory Rate - continuous

C. Hematological Measures (at -15 and 0)

1. Blood P_{O_2} - arterial and venous (in triplicate)

2. Microhematocrit - venous (in duplicate)

3. Cholinesterase activity (as nmoles Acetylcholine hydrolyzed/ml whole blood/hr) - arterial (in triplicate)

II. Drug Infusion for 15 minutes - Observations as described in I: A, B, and C (drug infusion time = 0 - +15 minutes)

A. Measures A. and B. from section I, plotted at +7.5 and +15 minutes with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, and 4 from section I-C. at +7.5 and +15 minutes; measure 5 from section I-C. at +15 min

III. Observation period - 120 minutes post-drug

A. Measures A. and B. from section I., plotted at 10 minute intervals beginning at +25 min and continuing through +135 min - with expanded record (25 and 100 mm/sec)

B. Measures 1, 2, 3, 4, and 5 from section I-C. taken at +25, +45, +85, and +115 min

EXPERIMENTAL GROUPS

			DOSE	PUREBRED BEAGLES (NUMBER)
1.	Pyridostigmine Br	0.5 mg/kg	(1.9 µmoles/kg)	6 dogs
2.	Pyridostigmine Br	2 mg/kg	(7.7 µmoles/kg)	6 dogs
3.	Pyridostigmine Br	5 mg/kg	(19.1 µmoles/kg)	6 dogs
4.	Vehicle Control Solution	Normal saline		6 dogs

All doses were given in a total volume of 29 ml given at a volume-rate of 1.91 ml/min for 15 mins. The cannula was loaded with drug prior to onset of the infusion to insure accurate drug delivery. The volume of the Mestinon® solution in the vial was measured gravimetrically at 21°C to establish the desired dose concentration and saline was added q.s. to 29 ml for the set infusion rate.

Nota Bene

The conduct of these studies comply with the GOOD LABORATORY PRACTICES (GLP) regulations as published in the Federal Register, Volume 43 (247), 22 December 1978, Part II, pp 59,986-60,020 and all subsequent addenda.

RESULTS

A summary of all baseline (time zero) data appears as absolute values in Table 1. Data on range of baseline values, range of responses and time of extreme values are given in table 4. The raw experimental data for each dog are given in tables of Appendix E.

RESPIRATORY FUNCTION

TIDAL VOLUME (TV)

Baseline values for tidal volumes for the four experimental groups at time zero ranged from 142±13 to 395±105 ml/breath (Table 1). The range of baseline values was large in the control and low dose groups (Table 4) and was, in general, inversely related to the baseline respiratory rate of each dog. These data serve to point out the considerable variability for this measurement. Large variations in the response were noted in the low dose group from +35 to +135 min.

The low dose may have produced a slight depression in TV of about 10%, with the greatest effect being noted 10 min following the infusion (Fig. 1). The higher doses produced a more marked depression during drug infusion. This reduction was of similar magnitude (25-30%) for both the mid and high doses; the effects persisted through +55 minutes of the observation period. The time to peak response was dose-related, occurring earliest for the high dose and latest for the low dose (Fig. 1).

RESPIRATORY RATE

Baseline values for respiratory rate varied from 8.9±2.5 and 14.4±6.6 breaths/min in the various groups (Table 1). The greatest

variability in baseline values was in the low dose group (Table 4). One dog (Table E-10) exhibited an unusually high baseline rate (44/min), which did not appear to be a function of anesthesia. This dog had a low tidal volume.

The low dose did not alter respiratory rate (Fig. 2). Both the middle and high doses produced approximately a 100% elevation in rate. There was considerable variability in responses within the mid dose group, particularly at +7.5, 15 and 55 min. This elevation in rate persisted and waned slowly over the experimental period. One dog (Table E-16) exhibited high rates at these points.

MINUTE VOLUME

Minute volume is the product of average tidal volume and respiratory rate. Mean baseline values ranged from 1.3±0.2 to 2.9±0.5 l/min for the groups (Table 1). This variable was not affected by the low dose (Fig. 3). The mid and high doses produced similar elevations in minute volume of 35% to 50%. Wide fluctuations in minute volume over time were particularly evident for the middle dose due primarily to one dog (Table E-16). Values fell slowly toward control. Variability to this calculated measure was largely due to variability in respiratory rate.

COMPLIANCE

Absolute baseline values for compliance ranged from 23.5±3.7 to 53±10.3 CU (Table 1). There was considerable variation to individual values; in general, dogs with high tidal volumes exhibited high compliance values.

Only the high dose infusion had any definite effect upon airways compliance (Fig. 4). A gradual depression of 35-40% occurred over the 45

minute period beginning with the infusion. Compliance remained depressed over the remainder of the observation period in the high dose group. A small (10%) depression of questionable significance occurred with the infusion of the low and mid-dose.

Response ranges in all groups were extensive, but particularly in the high dose group where one dog exhibited a marked rise in compliance at +65 and 75 min (Table E-22). Tidal volume was briefly elevated over these time points. Increased variation is to be expected when toxic doses are given that seriously alter physiological functions.

RESISTANCE

Baseline resistance values ranged from 2.7±0.9 to 5.5±1.5 resistance units (Table 1). Within each group, there was a considerable range of values (Table 4).

Airways resistance was elevated in a dose-related manner (Fig. 5). By far, the most prominent rise (≈1000%) was produced by the high dose after the end of infusion (+25 and 35 min). Considerable variability was noted in the magnitude of these peak responses (Figure 5, Tables E-19, 24 and Table 4); it was most prominent in the high dose group. Resistance decreased only partially throughout the experimental period as the mean values at the end of the observation period were still elevated ≈ 300% above control levels. The mid-dose produced a rise of ≈ 220% that remained throughout the observation period. A gradual elevation of approximately 200% occurred at ≈ 40-70 min with the low dose; a great deal of this rise and variability was due to an exceedingly marked rise in resistance at +45 min in one dog (see Figure 5, Tables E-11 and 4).

Values tended to drop toward control values toward the end of the observation period.

BLOOD CHEMISTRIES

Baseline arterial PO_2 ranged from 72.8±7 to 85±5 mmHg (Table 2). Values in the high dose group were surprisingly low (Tables E-19-24). Oxygen tension in arterial samples was not generally affected by pyridostigmine (Table 2). However, a small drop in PO_2 appeared to occur at +7.5 min in the 5.0 mg/kg group and mean values over the entire experimental period were lower in this group. Baseline venous PO_2 ranged from 45±4 to 52±3 mmHg. The only apparent changes in venous PO_2 was a rise (14 mmHg) which occurred at +15 min in the high dose group.

Baseline venous blood hematocrit ranged from 39±2 to 42±3 percent cells. Hematocrit appeared to rise with the infusion of pyridostigmine. This rise was most evident in the high dose group. About a 20% rise occurred at +15 min and remained elevated throughout the whole experimental period (Table 2).

CARDIOVASCULAR FUNCTION

SYSTOLIC BLOOD PRESSURE

Baseline systolic blood pressure ranged from 134±7 to 161±15 mmHg in the four groups (Table 1). The middle and high doses elevated systolic blood pressure modestly (10%) during the infusion (Fig. 6). Immediately following the infusion, pressure fell about 10% below baseline values in these two groups over the next 20 minutes, then leveled toward control values. The low dose did not alter systolic blood pressure. Values in the control group rose abruptly at +85 min and remained elevated. This rise was mainly due to a 50 mmHg rise in one dog (Table E-4); cardiac output rose over 40% from +75 to 85 min in the dog.

DIASTOLIC BLOOD PRESSURE

Baseline diastolic blood pressure ranged from 97±5 to to 118±12 mmHg for the four groups (Table 1). The high dose produced a fall in pressure of approximately 25% at 35 minutes (Fig. 7). No important drop was evident for the low and middle dose. The reduction in pressure by the high dose persisted through +75 minutes; pressure then climbed toward control values. Large variability was noted in response of the high dose; one dog exhibited a marked anomolous rise at +7.5 min of unknown etiology (Table E-19); several others had marked drops in pressure during and just after dose administration (tables E-20, 22, 23). The abrupt rise in the control group at +85 min was due to response of one dog (Table E-4), as noted above for systolic pressure.

HEART RATE

Baseline heart rate of the groups ranged form 139±14 to 157±10 bpm (Table 1). Heart rate decreased in a dose-related manner during the

infusion of the mid and high dose (Fig. 8). The maximum decrease with high dose was approximately 45% at the end of infusion. The middle dose produced a 20% fall in rate at +15 and 20 min; a secondary fall in rate appeared related to a spontaneous fall also noted in the control group over the duration of the experiment. A slight fall in heart rate (= 10%) may have occurred with the low dose; the responses within the group were quite variable with one showing a prominent fall of 35% (Table E-12). Heart rate in all groups returned towards control values following the infusion with the high dose values remaining depressed longest.

CARDIAC OUTPUT

The baseline values for cardiac output ranged from 1.56 ± 0.25 to 2.3 ± 0.19 L/min. During the infusion period, cardiac output increased to all drug doses (Fig. 9). The peak increase for the middle and high dose occurred at the midpoint of the infusion. Cardiac output then fell over the remaining time period. The output in the high dose group rose less and fell more (25%) than the mid dose group. One mid-dose dog exhibited a very marked rise at + 7.5 min (Table E-16) accounting for the large variability. The low dose caused a minor increase in cardiac output in one dog over the entire infusion period.

LEFT VENTRICULAR dP/dt

dP/dt is an estimate of left myocardial contractility. Baseline dP/dt varied from 1837 ± 224 to 2395 ± 323 mmHg/sec among the groups (Table 1). The middle and high doses produced a 20% increase in left ventricular dP/dt by the end of the infusion period (Fig. 10); responses in the high dose group at + 15 and + 25 min were particular variable. A prominent rise in one dog (E-19) and a fall in another (E-20) were mainly responsible for this variability. These responses waned to control values by + 35 minutes. Left ventricular dP/dt was not affected by the low dose.

MEAN PULMONARY ARTERY PRESSURE

Baseline values ranged from 10.7 ± .6 to 11.6 ± 1.1 mmHg in the groups. Mean pulmonary artery pressure increased in a dose-related manner during the infusion with a maximum increase of approximately 45% at the high dose (Fig. 11). The mid dose peak response at + 15 min was similar to that of the high dose but the response at + 7.5 min was definitely smaller than with the high dose. Pressure decreased to control values over the 20 minutes following the infusion. An abrupt increase in control values at + 85 min was due to response of one dog (Table E-4) which also exhibited rises in cardiac output, systolic and diastolic systemic pressures.

PULMONARY VASCULAR RESISTANCE (PVR)

Baseline values for pulmonary vascular resistance ranged from 5.0±0.4 to 8.6±2.3 mmHg/l/min (Table 1). This variable increased during both the mid and high dose infusions (Fig. 12). The mid dose did not increase PVR until +15 minutes; variability within this group is largely due to one dog which exhibited an abrupt increase in PAP and drop in CO at +15 min (Table E-15). Values in the mid dose group returned toward control rapidly; the response to the high dose persisted until about +75 minutes. The low dose did not alter PVR during the first hour; PVR tended to paralled the rise noted in the control group.

PULMONARY WEDGE PRESSURE (PWP)

Baseline PWP ranged from 2.9±0.5 to 3.5±0.7 mmHg (Table 1). All doses produced an elevation in PWP (Fig. 13). The low and mid doses produced similar peak increases of about 3 mmHg at the end of infusion. The high dose caused an increase in pressure of approximately 6 mmHg. As with PVR, PWP in the control group tended to rise over the experimental period; however, a large contributor to this rise and the variability was the response of one dog at +85 min (Table E-4).

BLOOD CHOLINESTERASE (Che) ACTIVITY

Baseline cholinesterase activity varied from 178.4±21 to 243.4±36 nMoles of acetylcholine hydrolyzed/ml of whole blood/hr. Cholinesterase activity dropped during the infusion in a dose-related manner; the nadir appears to occur at the end of the infusion (Fig. 14). The lowest dose caused a 40% fall in activity while the highest dose produced a 60% inhibition of enzyme activity. The mid dose produced a 50% depression.

A partial rebound of activity of 10-20% occurred in all drug groups during the 10 minute period following the infusion, but values for all doses remained consistently depressed for the remaining time period. Cholinesterase activities in the mid and high dose were similarly depressed.

ELECTROCARDIOGRAPHIC EFFECTS

Pre-drug values for P-R interval varied from 80±2 to 91±4 mSec in the four experimental groups (Fig. 15, also see Tables 1 and 3 and Fig. 16). During the mid and high dose rate infusions, P-R interval increased by about 15-20 mSec. The rise was more rapidly attained for the high dose group; the peak occurred by +7.5 min. Values in these groups fell slowly after the infusion. There may have been a slight increase in P-R interval to the low dose.

Pyridostigmine at 0.5 and 2.0 mg/kg caused no ectopic beats or changes in rhythm. In one dog, the high dose of 5 mg/kg produced some short runs of ectopic beats during the infusion and these decreased and disappeared over the 30 minutes following the infusion. A second dog at 5 mg/kg had scattered ectopics before the infusion of pyridostigmine which were maintained with no obvious change in frequency during and for an additional 30 minutes after the dose and then disappeared. It is likely that this case was unrelated to the pyridistigmine dose.

The drug had no effects on the P-wave, the T-wave, or the QRS configuration (Table 3). The P-R interval was increased slightly by the 5 mg/kg dose as might be expected, although it did not approach the upper limit of normal of 0.13 sec. (Ettinger & Suter, 1970). The major alteration was an increase in the Q-T interval which appeared at the end of the

infusion and persisted throughout the observation period at the 5 mg dose. The baseline Q-T interval was on the upper edge of normal values and increased with the 5 mg dose by 28% at 15 minutes and 38% at 60 and 120 minutes. However, when the Q-T interval was corrected for heart rate the increase dropped to 0% at 15 minutes, 11% at 60 minutes, and 17% at 120 minutes. Pyridostigmine apparently has some effect to slow repolarization of the ventricle, and this action may contribute to the occasional arrhythmias seen. Only control group and high dose group electrocardiograms are compared in Table 3 as low and mid dose groups lacked consistent changes.

Figure 16 depicts representative tracings from two dogs in each group at time zero and +15 minutes.

OTHER OBSERVATIONS

Mucous production and defecation occurred in every dog at the high dose. The quantity of mucous ranged from 60 mls to 110 mls. Both defecation and mucous production usually occurred during or just following the infusion. Responses to the mid dose were somewhat varied. Two dogs in this group showed no defecation and no significant mucous production. Mild mucous production (≈20 mls) and defection occurred in another two dogs of the mid-dose group. In the two remaining experiments one dog had mild mucous and no defecation, the other had defecation and no mucous production. The low dose caused no measurable mucous production. In two instances at the low dose, defecation occured immediately following the infusion.

Lungs excised from control and high dose group dogs demonstrated similar abilities to float in containers of water. Cross-sectional cuts of these lungs did not reveal any gross differences in mucous or fluid

content, at least in middle sized bronchioles. Mucous content of larger, secondary branch bronchioles was obviously greater in the high dose as opposed to the control group.

Summary of CARDIOVASCULAR COMPOSITE DATA (See Appendix D)

Heart rate was reduced in response to pyridostigmine in a dose related fashion. It, in some ways, mirrored the rise in P-R interval caused by this drug; possibly because both these actions were due to enhancement of vagal function; the fall in diastolic pressure correlated with the fall in heart rate. Even in spite of reductions in heart rate, cardiac output was maintained or even augmented during the infusion period. Stroke volume rose in a dose-related fashion during drug infusion. This rise in cardiac output during drug infusion was associated with an increase in cardiac contractility, LV dP/dt. Rises in both cardiac contractility and output corresponded temporally to the increases in systolic and arterial pulse pressures.

In the pulmonary vascular bed, pyridostigmine infusion increased arterial pressure at all dose-rates. Vascular resistance was also elevated in a parallel fashion. It appears that a strong direct pulmonary vasoconstriction occurred, since cardiac output was maintained or even augmented during this period. Since pulmonary wedge pressure was also elevated, this suggests that an active constriction of the pulmonary veins and/or capillaries occurred.

Summary of RESPIRATORY COMPOSITE DATA (See Appendix D)

Respiratory rate was variably elevated during the mid and high dose-rate infusion, and during this period, the average tidal volume was reduced by about 25%. Minute volume, the product of respiratory rate times tidal volume, was elevated by both the mid and high dose; the greater contributor to this response and variability was rate. Minute volume and respiratory rate were

maintained at a level higher than control for most of the observation period. Airway resistance was markedly elevated by the high dose of pyridostigmine. The rise in airways resistance was mirrored by a drop in airways compliance; but these two responses are probably not causally related.

DISCUSSION

Pyridostigmine is a reversible inhibitor of choline esterase, the enzyme which destroys and stops the actions of the neurotransmitter, acetylcholine. In general, the pharmacological properties of anticholinesterase agents, like pyridostigmine, can be predicted merely by knowing those loci where choline is released physiologically by nerve impulses and the responses of corresponding effector organs. However, the many and diverse locations of cholinergic synapses increase the complexity of the response. Potentially, an anticholinesterase agent which can traverse lipid membrane barriers can produce all of the following effects: (1) stimulation of muscarinic receptor responses at autonomic effector organs; (2) stimulation, followed by depression or paralysis, of all autonomic ganglia and skeletal muscle (nicotinic actions); and 3) stimulation, with possible subsequent depression of cholinergic receptor sites (primarily muscarinic) in the CNS (Taylor, 1985).

Cholinesterase activity in blood was reduced in a dose-related fashion by pyridostigmine. Reduction in activity was greatest at the end of drug infusion. There was a partial (10-20%) recovery of enzyme activity over the 10 minute period immediately after the end of drug administration. A maintained reduced state of inhibition was then apparent for the rest of the experimental period. This partial recovery may have been due to a redistribution of pyridostigmine from blood to other body compartments.

Cardiovascular Effects

The low dose of pyridostigmine was chosen to have only minor effects and this was certainly true with regard to the cardiovascular system. Only on heart rate and perhaps briefly on diastolic blood pressure could one see a

possible effect of the 0.5 mg/kg dose. Heart rate proved to be the most sensitive and dependable indicator of the presence of pyridostigmine, showing a progressive decrease as the dose was raised. At the high dose, the duration of the bradycardia was such that the heart rate was still not back to the control value after 120 minutes. The contractility of the ventricle (dP/dt) was increased for a short time by the two higher doses; this may be partly due to an increase in and ventricular filling related to the reduction in heart rate and resistance. Resultant stretching of the heart could enhance contractions through the Starling mechanism. This proposition is certainly substantiated by the values of stroke-volume which rose in a dose-related manner during drug infusion and mirrored the dose related falls in heart rate. The simultaneous occurrence of a transitory increase in cardiac output during the infusion may also be explained in this same way. Nicotinic stimulation of sympathetic nervous ganglia can not be discounted as a means of enhancing cardiac contractility; however, the bradycardia observed during this period does not corroborate this possibility. The fall in blood pressure, which was more prominent with diastolic than systolic, is no doubt related to the cholinergic actions of pyridostigmine to slow heart rate and dilate peripheral blood vessels. The cholinergic effects were also seen in a mild increase in conduction time across the A-V node (increase in P-R interval) and a somewhat greater interference with repolarization in the ventricle. One animal developed bouts of ectopic beats for a short time following the high dose which may have been associated with the change in conduction and repolarization of the heart.

Effects of pyridostigmine on the pulmonary circulation differed from that seen in the peripheral circulation. Increases in pulmonary artery pressure and vascular resistance have been described in response to pyridostigmine (Taylor, 1985 and Ehrlich et al. 1985). The nature of this response is not clear. The increase in pulmonary wedge pressure, an estimate of left atrial pressure, was an unexpected observation since cardiac contractility and output were maintained after pyridostigmine administration. This rise in wedge pressure which also reflects pressures in the pulmonary capillaries and veins may be a consequence of constriction of these structures.

Blood PO_2 gases were generally not affected by administration of pyridostigmine. An exception may have been the increase in venous blood PO_2 noted during the high dose infusion. A possible reason for this effect could be a depression in O_2 utilization by peripheral body tissues caused by pyridostigmine. A rise in hematocrit which was particularly evident at the end of the high-dose infusion may have been due to dehydration as a result of loss of fluid through marked mucous production during this period. Another possible explanation is that contraction of the spleen through stimulation of celiac sympathetic ganglia or through reflex sympathetic stimulation pushed cell-rich blood into the circulation.

Respiratory Effects

Pyridostigmine produced prominent tachypnea at the highest doses which persisted throughout the experimental period. Central nervous stimulation within the respiratory center is possibly responsible (Taylor, 1985, Borland et al., 1985). The quaternary nitrogen in its structure makes this agent relatively lipid insoluble and less able to traverse the blood-brain barrier; however, respiratory function neural pathways are near the area postrema, a region devoid of the blood-brain barrier. The drop in tidal volume observed

at these doses is probably the passive consequence of a shorter respiratory cycle. Whether or not a paralysis of chest wall skeletal muscles was involved in the depression of tidal volume is uncertain, but unlikely, since minute volume was maintained or even augmented at the doses tested. Lack of blood gas changes confirmed that adequate ventilation was occurring.

Airways resistance was elevated by administration of pyridostigmine; peak responses were dose related. The increased airways resistance was particularly notable in the high dose group (≈1000%). The rise in resistances was probably a consequence of a buildup of acetylcholine, an effective bronchoconstrictor. Vagal acetylcholine-containing fibers are known to course to the bronchiolar smooth muscle (Sanford, 1976). Additionally, acetylcholine of cardiac origin would be expected to spill-over into the pulmonary circulation in the presence of acetylcholinesterase inhibition. An additional contributor to the rise in airways resistance is probably an increase in bronchial secretions and thus some luminal obstruction (Slonin and Hamilton, 1971). Mucous secretions in the oral and nasal cavities were certainly profuse in the high dose group. Although we saw no gross evidence for increased mucous secretion in small-size bronchioles at autopsy, we cannot exclude the possibility of enhanced mucous secretion from bronchiolar glands.

Airways compliance was definitely depressed by the high dose of pyridostigmine. Deficiency or dilution of pulmonary surfactant are prominent causes for a loss in compliance (Slonin and Hamilton, 1971). This lecithin-containing substance keeps alveoli from collapsing and allows larger increases in lung volume to occur without a rise in pressure. Dilution of surfactant with bronchiolar mucous secretions may be responsible for the fall in compliance.

Previous experiments performed in mongrel dogs given these same dose-rates of pyridostigmine revealed that responses were generally similar to those observed in beagles. Notable exceptions were that airways resistance was not raised as much in mongrels and that P-R interval was lengthened more in mongrels.

CONCLUSION

Pyridostigmine in doses of 0.5 to 5 mg/kg i.v. in the dog produced a dose-related inhibition of plasma cholinesterase activity. All of the cardiopulmonary changes can be related to the effects of Pyridostigmine directly on cholineserase activity or through effects subsequent to cholinesterase inhibition. Heart rate, which was marked reduced, and airways resistance, which was markedly elevated, appear to be the variables most affected. Other changes in cardiovascular and pulmonary function may be considered as consequences of these primary events.

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APPENDIX A
LEGEND OF TERMS USED

Abbreviation	Definition
TV-ml/breath	Tidal Volume-ml/breath
Resp. rate	Respiration Rate-breaths/min
MV-L/min	Minute Volume-liters/min
C-cu	Respiratory Dynamic Compliance-compliance units
R-ru	Respiratory Dynamic Resistance-respiratory units
SBP-mmHg	Aortic Systolic Blood Pressure-mmHg
DBP-mmHg	Aortic Diastolic Blood Pressure-mmHg
ABP-mmHg	Aortic Blood Pressure
HR-beats/min	Heart Rate-beats/min
C.O.-L/min or CO	Cardiac Output-liters/min
SV-ml/beat	Stroke Volume-ml/beat
dP/dt-mmHg/sec	Acceleration of pressure, a quantitative expression for defining contractility of the heart
PWP-mmHg	Pulmonary Wedge Pressure - an estimate of left atrial pressure
PAP-mmHg	Pulmonary Artery Pressure
PVR-mmHg/l/min	Pulmonary Vascular Resistance
A-Po_2-mmHg	Arterial Blood Oxygen Tension
V-Po_2-mmHg	Venous Blood Oxygen Tension
Hct-% cells	Hematocrit-% Red Blood Cells
Ache	nmoles acetylcholine hydrolyzed/ml of whole blood/hr

Appendix B

Tables 1, 2, 3 and 4

Note: All variation bars represent S.E.M.

TABLE 1

Baseline Values Table 1 (time zero) - Beagles

	Group			
	0.5	2.0	5.0	Control
TV Ml/breath	224±64	142±13	274±62	395±105
Resp. rate (breaths/min)	14.4±6.6	9.3±1.6	8.9±2.5	10.7±3.3
M.V. (L/min)	2.2±0.4	1.3±0.2	1.9±0.3	2.9±0.5
C (CU)	44±12.3	23.5±3.7	45.1±10.8	53.0±10.3
R (RU)	3.4±0.8	2.8±0.5	5.5±1.5	2.7±0.9
SBP mm Hg	161±15	147±10	159±10	134±7
DBP mm Hg	118±12	112±9	117±9	97±5
HR beats/min	157±10	149±5	144±10	139±14
SV ml/beat	11.9±1.6	10.6±1.7	16.3±1.5	13.4±2.0
C.O. L/min	1.85±0.28	1.56±0.25	2.30±0.19	1.79±0.19
dP/dt mm Hg/sec	2395±354	2336±214	2210±278	1837±246
MPAP mm Hg	11.2±1.9	11.6±1.5	11.5±1.1	10.7±0.6
PVR mm Hg/l/min	6.0±0.7	8.6±2.3	5.0±0.4	5.8±0.6
PWP mmHg msec	2.92±0.5	3.17±0.6	3.5±0.7	3.08±0.6
P-R interval msec	86±4	80±2	84±5	91±4
Ache (nmoles acetylcholine hydrolyzed/ml of whole blood/hr)	243±36	178±21	231±32	20±31

Values given are mean ± S.E.M. (N=6, each group)

TABLE 2
Blood Chemistries - Beagles

Arterial Blood PO_2 (mm Hg)

Group	Time-min. -15	0	7.5	15	25	45	85	115
Control	75±5	84±5	80±6	81±6	84±6	87±5	74±10	89±12
0.5	75±5	75±8	75±8	84±7	80±5	81±7	85±8	84±7
2.0	70±7	85±5	83±5	75±8	85±7	94±5	90±5	91±10
5.0	73±4	73±7	53±7	66±8	62±6	65±4	75±2	72±5

Venous Blood PO_2 (mm Hg)

Control	54±5	52±3	47±5	45±5	46±5	47±3	45±5	47±7
0.5	44±5	45±5	47±3	47±6	48±5	43±6	45±6	42±5
2.0	47±5	51±4	53±5	52±6	52±5	48±5	38±3	45±5
5.0	48±5	45±4	47±5	61±8	51±5	51±2	52±3	49±3

Hematocrit % cells

Control	40±3	39±2	36±6	38±2	41±3	40±0.5	38±1	37±2
0.5	41±3	42±3	40±2	45±1	44±2	42±2	41±3	41±2
2.0	41±1	41±1	41±3	46±3	45±3	43±6	42±2	42±2
5.0	40±1	42±1	49±1	52±1	53±1	53±2	51±2	50±2

Values given are mean ± 1 S.E.M.

TABLE 3

SUMMARY OF ECG CHANGES

Item	VEHICLE (Saline) 0	15	60	120 min	PYRIDOSTIGMINE - 5mg/kg 0	15	60	120 min
Heart Rate	139	134	128	118	142	78	92	103
P-wave	normal	normal	normal	normal	normal	normal	normal	normal
††P-R Interval	0.091	0.093	0.095	0.097	0.084	0.101	0.095	0.090
QRS Config	normal	normal	normal	normal	normal	normal	normal	normal
††QRS Width	0.036	0.037	0.037	0.038	0.033	0.033	0.034	0.033
††Q-T Interval	0.2	0.24	0.24	0.265	0.205	0.263	0.283	0.283
T-wave	normal	normal	normal	normal	normal	normal	normal	normal
U-wave	absent	absent	absent	absent	absent*	absent	absent	absent
Rhythm	N-S†	N-S	N-S	N-S	N-S**	N-S	N-S	

*U-wave present in 2 dogs of the pyridostigmine group at control time only

**One dog developed intermittant ectopics during and for approximately 30 minutes after infusion. A second dog had scattered ectopics before and after infusion or about 30 minutes.

†Normal sinus rhythm

††Intervals and width in msec.

TABLE 4

Range of baseline values at time zero, range of responses (percent of control) and time (min) of extreme responses.

Variable	Group	Baseline Range-Units	Range of Reponse (min)	
			Low	High
T.V. (ml)	Control	95-672	47(75)	159(135)
	0.5	68-480	49(95)	186(75)
	2.0	101-177	31(7.5)	118(15)
	5.0	106-451	52(15)	111(65)
Resp. rate (breath/min)	Control	3.1-24.0	48(105)	239(45)
	0.5	4.6-43.9	55(95)	320(85)
	2.0	5.2-15.1	46(135)	548(15)
	5.0	3.7-17.2	85(135)	297(105)
M.V. (L/min)	Control	1.7-4.6	59(75)	179(45)
	0.5	1.2-3.3	66(95)	158(85)
	2.0	0.8-1.9	61(105)	300(135)
	5.0	1.4-2.9	56(15)	300(75)
C (CU)	Control	17.7-85.6	65(125)	145(135)
	0.5	18.4-87.8	69(55)	113(105)
	2.0	13.0-36.4	67(15)	143(35)
	5.0	26.2-78.3	30(35)	190(65)
R (RU)	Control	0.5-5.4	45(125)	333(85)
	0.5	1.4-6.4	68(75)	1066(45)
	2.0	1.4-4.5	128(45)	657(55)
	5.0	1.1-8.8	24(95)	3254(35)

TABLE 4 (continued)

Variable	Group	Baseline Range	Range Response (time, min)
SBP (mmHg)	Control	121-160	81(45) - 130(85)
	0.5	115-190	71(95) - 111(15)
	2.0	120-177	68(115)- 140(115)
	5.0	130-180	60(7.5)- 133(15)
DBP (mmHg)	Control	84-115	78(45) - 115(115)
	0.5	92-149	70(95) - 109(7.5)
	2.0	91-147	61(115)- 136(135)
	5.0	88-140	50(35) - 122(135)
HR (beats/min)	Control	108-180	69(135)- 104(35)
	0.5	129-194	64(105)- 117(135)
	2.0	135-166	59(25) - 108(7.5)
	5.0	99-162	35(15) - 93(7.5)
SV (ml/beat)			
	Control	8.9-20.4	7.7(115)-30.0(125)
	0.5	7.9-17.5	5.7(135)-22.0(15)
	2.0	5.3-16.1	3.8(55) -19.9(7.5)
	5.0	11.0-19.4	11.0(0) -31.7(15)
C.O. (L/min)	Control	1.44-2.55	63(125)- 133(85)
	0.5	1.27-2.94	46(125)- 120(7.5)
	2.0	0.82-2.30	49(115)- 243(7.5)
	5.0	1.78-2.74	56(95) - 136(7.5)
LV dP/dt (mmHg/sec)	Control	1119-2580	71(125)- 137(115)
	0.5	1356-3363	52(95) - 119(25)
	2.0	1779-3078	50(65) - 142(15)
	5.0	1223-2850	66(35) - 152(105)

TABLE 4 (continued)

Variable	Group	Baseline Range	Range Response (time, min)
PAP (mmHg)	Control	8.7-12.1	39(75) - 161(115)
	0.5	4.9-17.2	52(95) - 157(15)
	2.0	9.5-15.9	40(135)- 178(115)
	5.0	8.2-12.7	19(75) - 220(15)
PVR (mmHg/l/min)	Control	4.39-8.40	48(75) - 166(135)
	0.5	3.86-7.77	66(75) - 209(125)
	2.0	3.32-18.20	45(55) - 234(25)
	5.0	3.69-6.02	26(75) - 210(15)
PWP (mmHg)	Control	2.0-5.5	1.0(135)- 15.0(95)
	0.5	1.5-4.0	1.5(7.5)- 12.0(15)
	2.0	1.0-3.5	1.0(75) - 11.0(15)
	5.0	1.0-5.0	1.0(15) - 13.5(125)
P-R Interval (mSec)	Control	78.5-103.5	78.5(7.5)- 113(75)
	0.5	71-95	71(7.5) - 106(135)
	2.0	73-88	68(135) - 134(7.5)
	5.0	74.5-105.5	75.5(15) - 1265(7.5)
Cholinesterase Activity (nmoles Ach hydrolyzed/ml blood/hr)	Control	181.5-304.7	67(15) - 105(15)
	0.5	152.1 374.5	43(15) - 97(25)
	2.0	100.3-283.5	37(15) - 75(25)
	5.0	67.7-272.1	26(15) - 77(115)

Appendix C

Variable Plots

Note: All variation bars represent S.E.M.

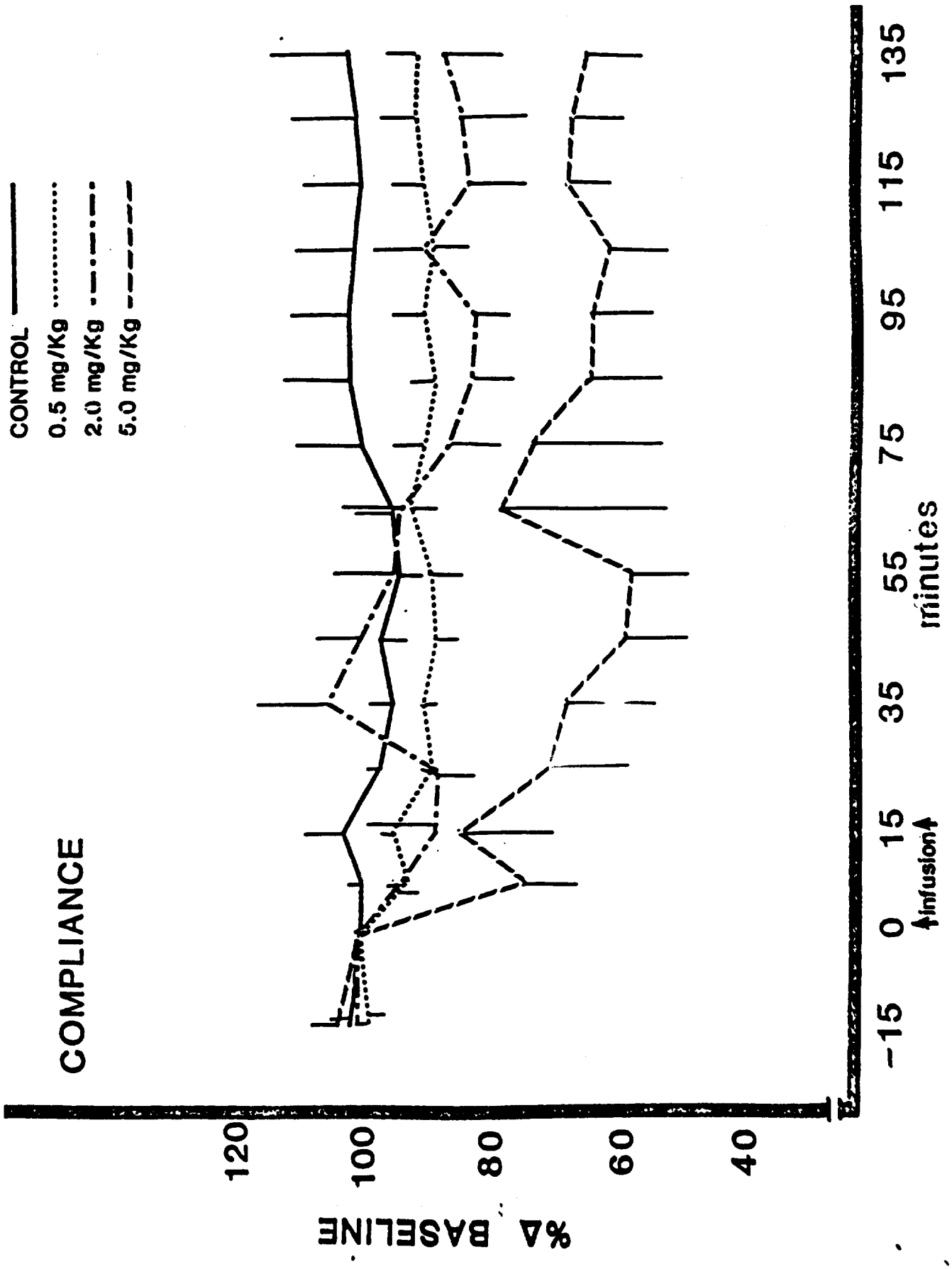

COMPLIANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
120
100
80
60
40
-15
0
15
35
55
75
95
115
135
minutes
Infusion

figure 6

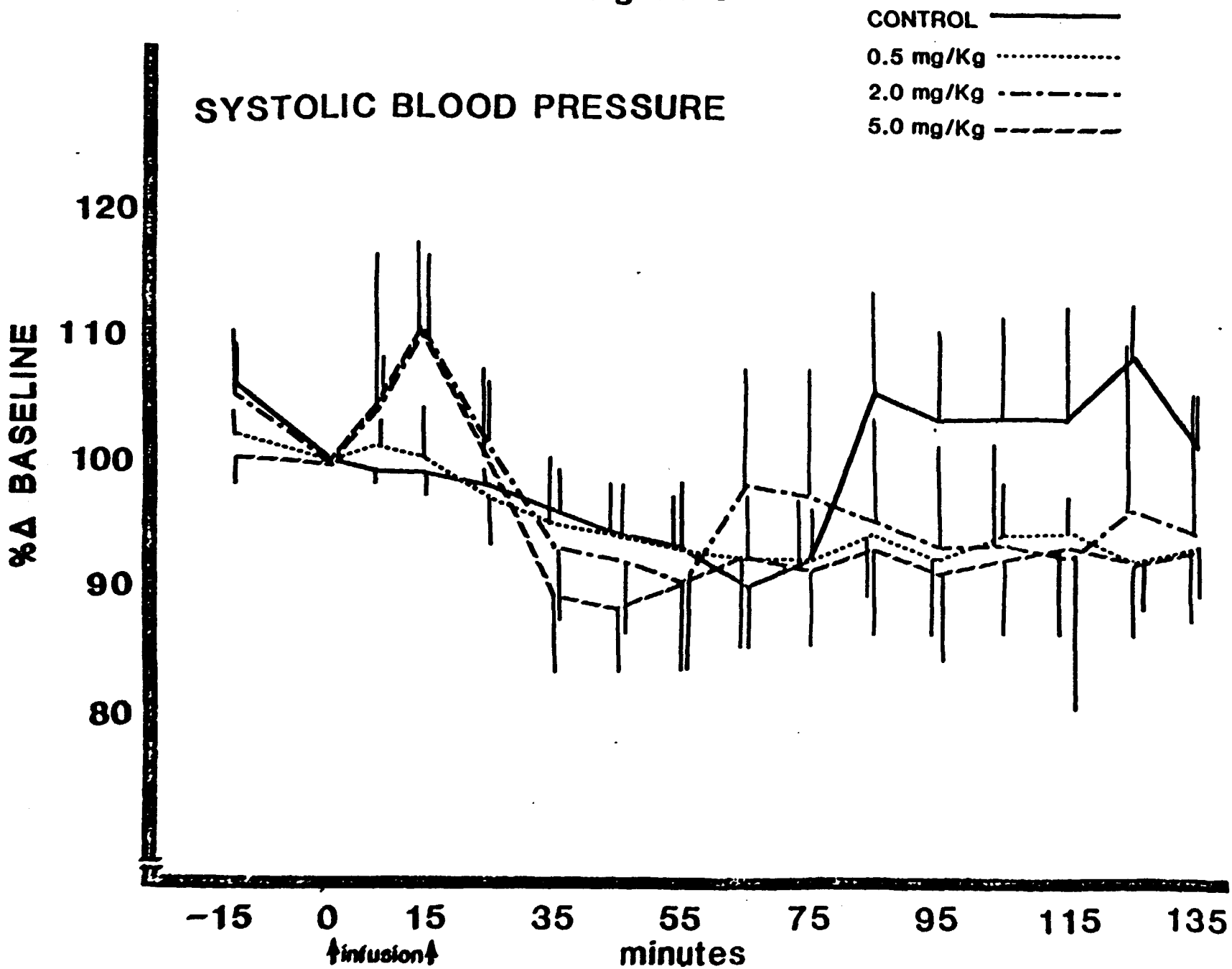

CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
SYSTOLIC BLOOD PRESSURE
%Δ BASELINE
120
110
100
90
80
-15
0
15
35
55
75
95
115
135
infusion
minutes

Figure 7

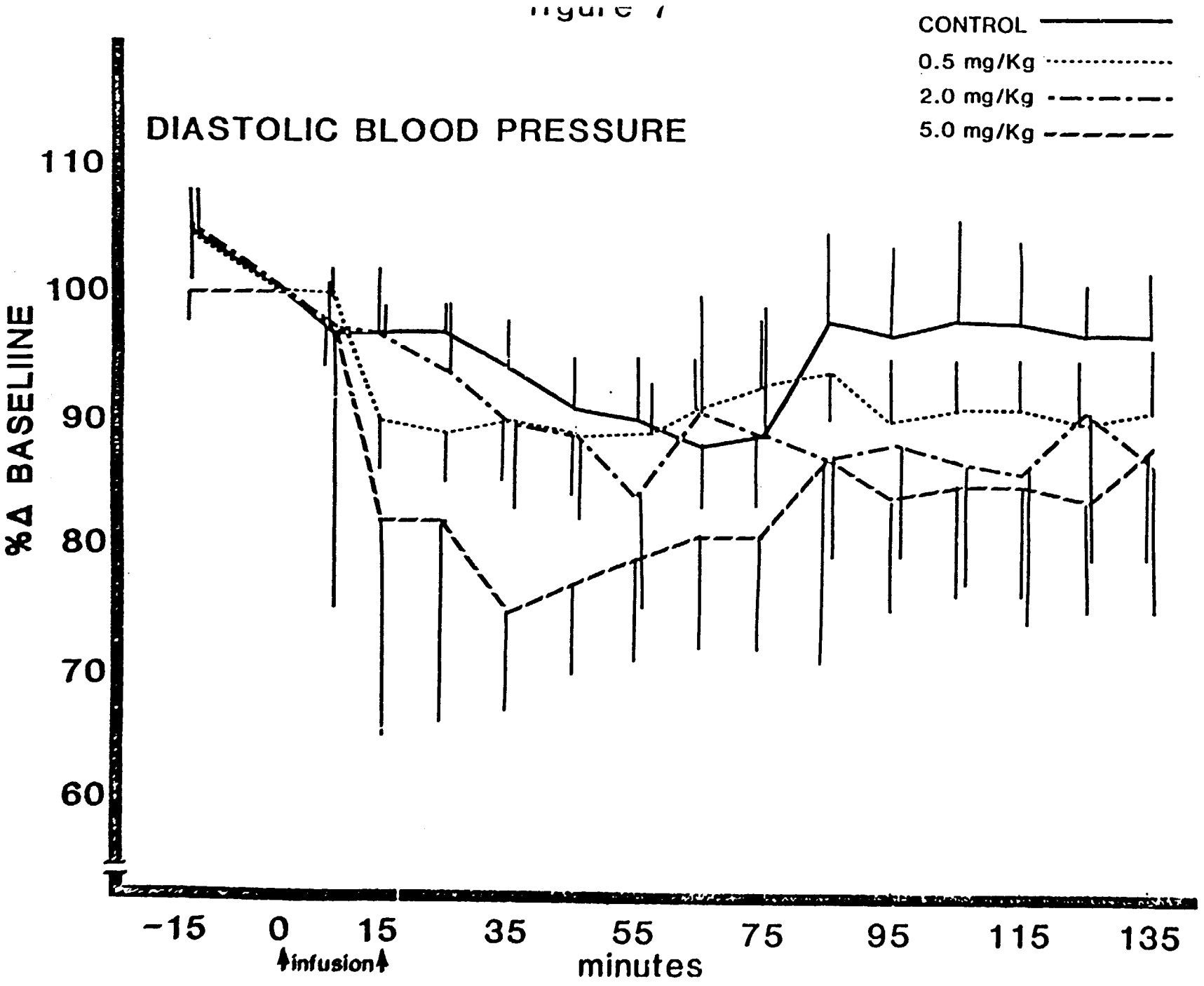
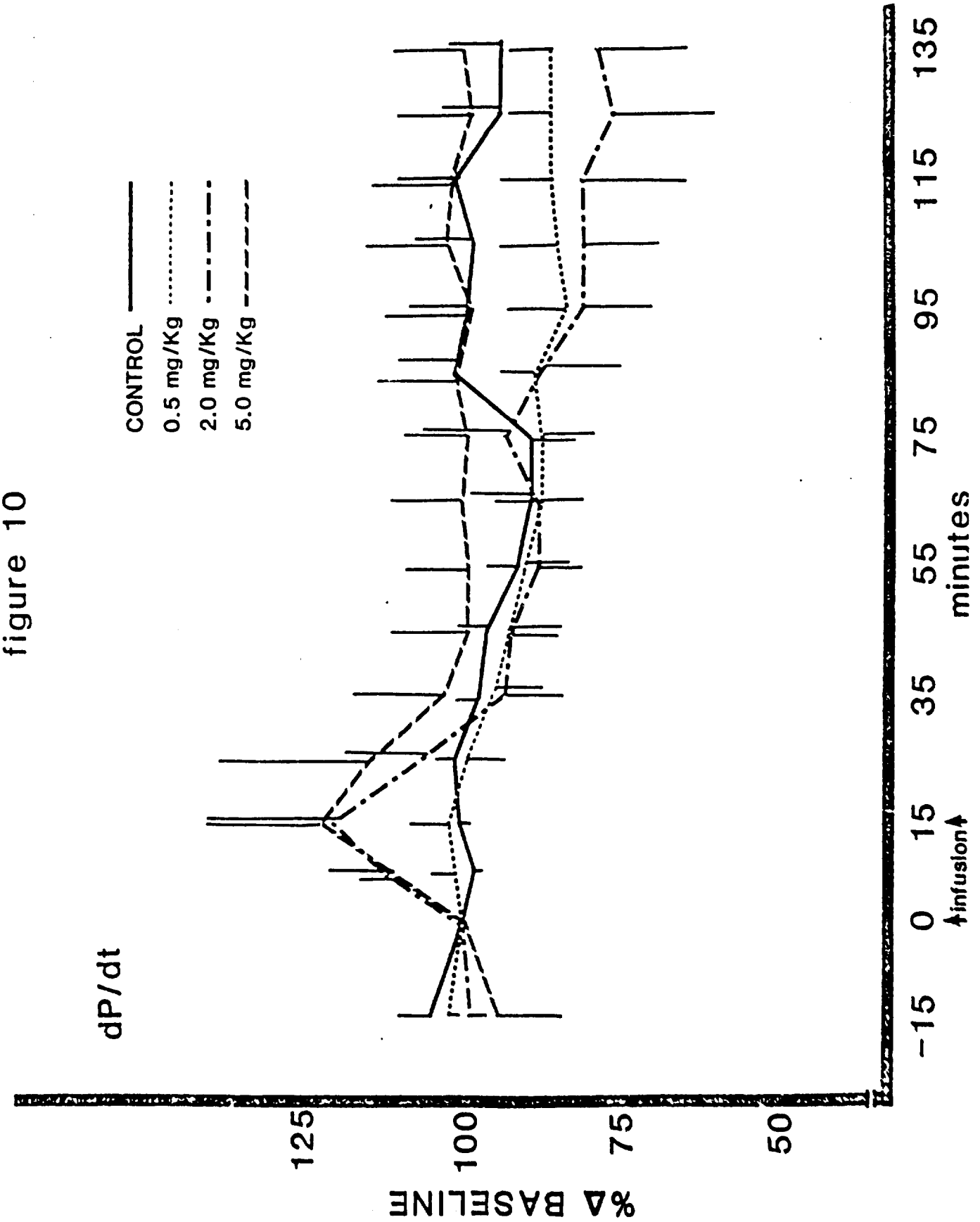

CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
DIASTOLIC BLOOD PRESSURE
%Δ BASELIINE
110
100
90
80
70
60
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 10


dP/dt
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
125
100
75
50
-15
0
15
35
55
75
95
115
135
minutes
infusion

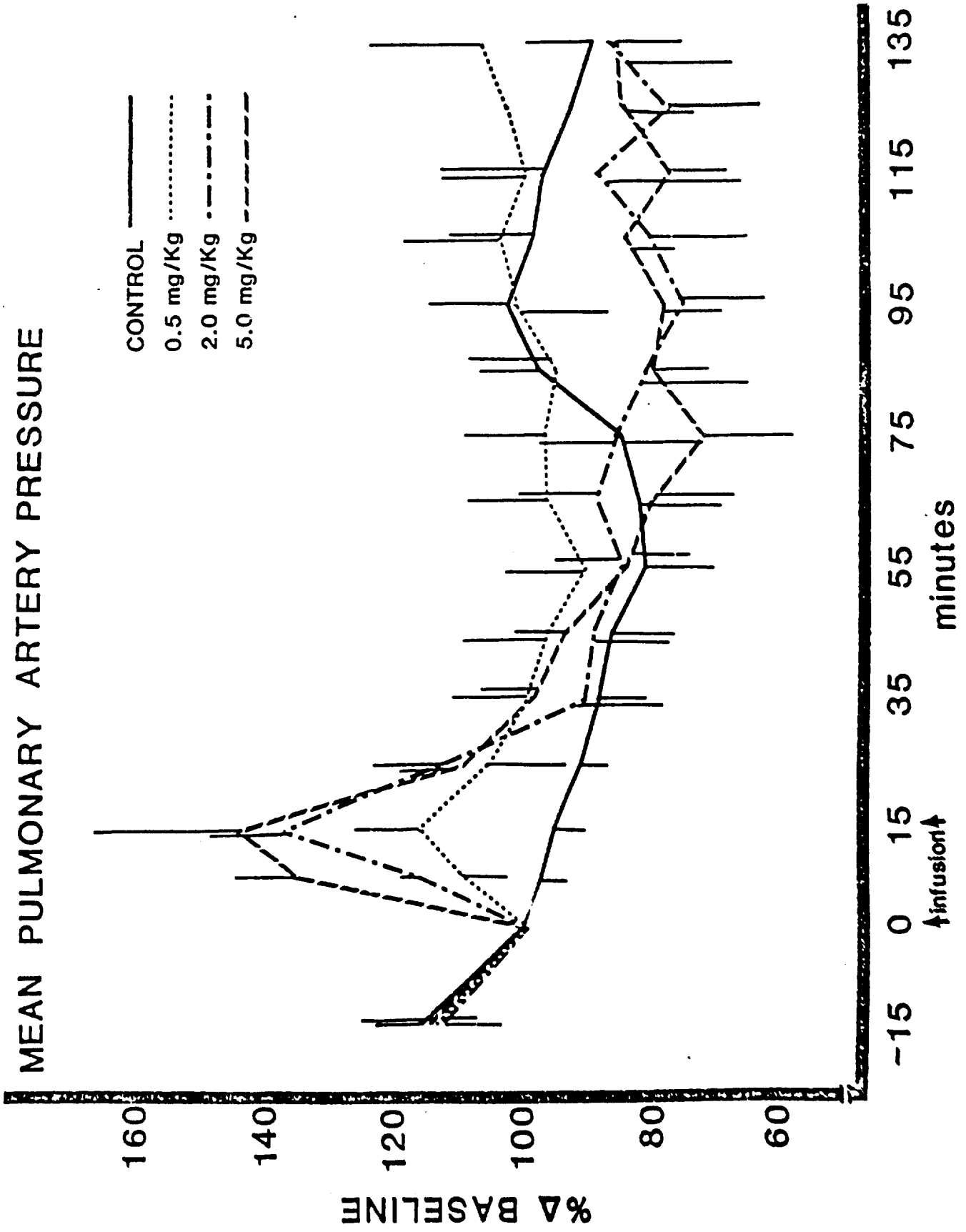

MEAN PULMONARY ARTERY PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
% Δ BASELINE
160
140
120
100
80
60
-15
0
15
35
55
75
95
115
135
minutes
infusion

figure 12

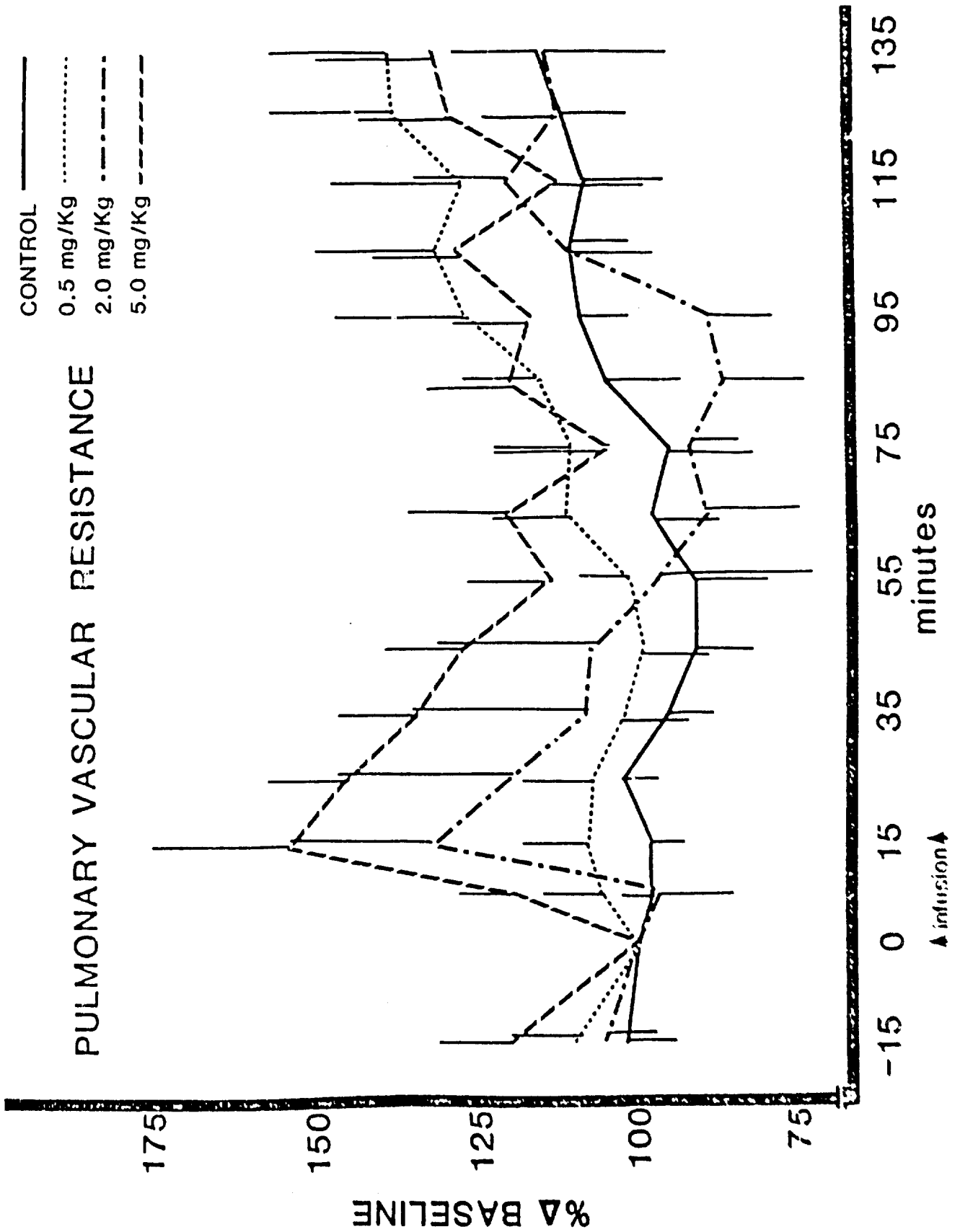

PULMONARY VASCULAR RESISTANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
175
150
125
100
75
-15
0
15
35
55
75
95
115
135
minutes
infusion

figure 13

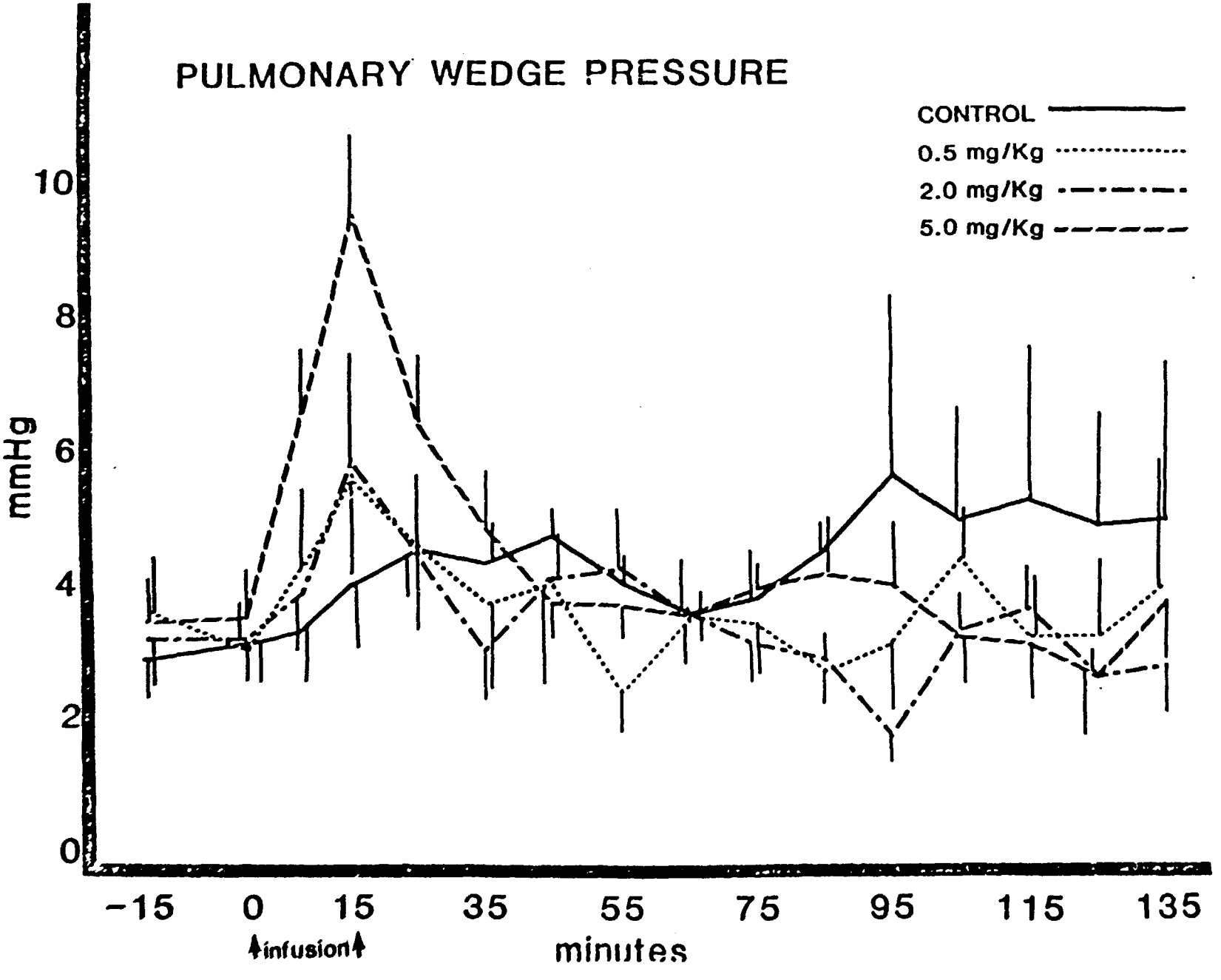

PULMONARY WEDGE PRESSURE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
mmHg
10
8
6
4
2
0
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 1

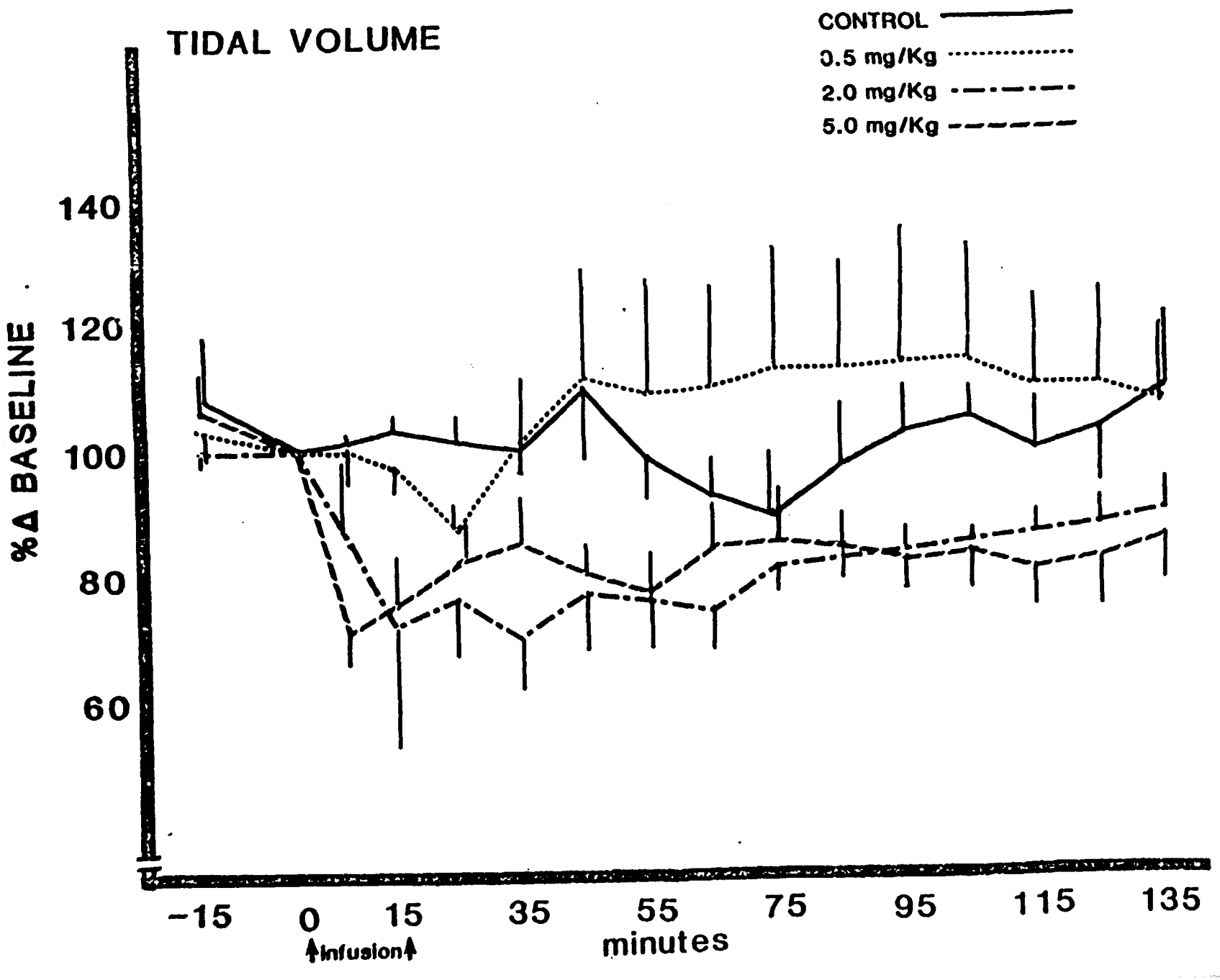
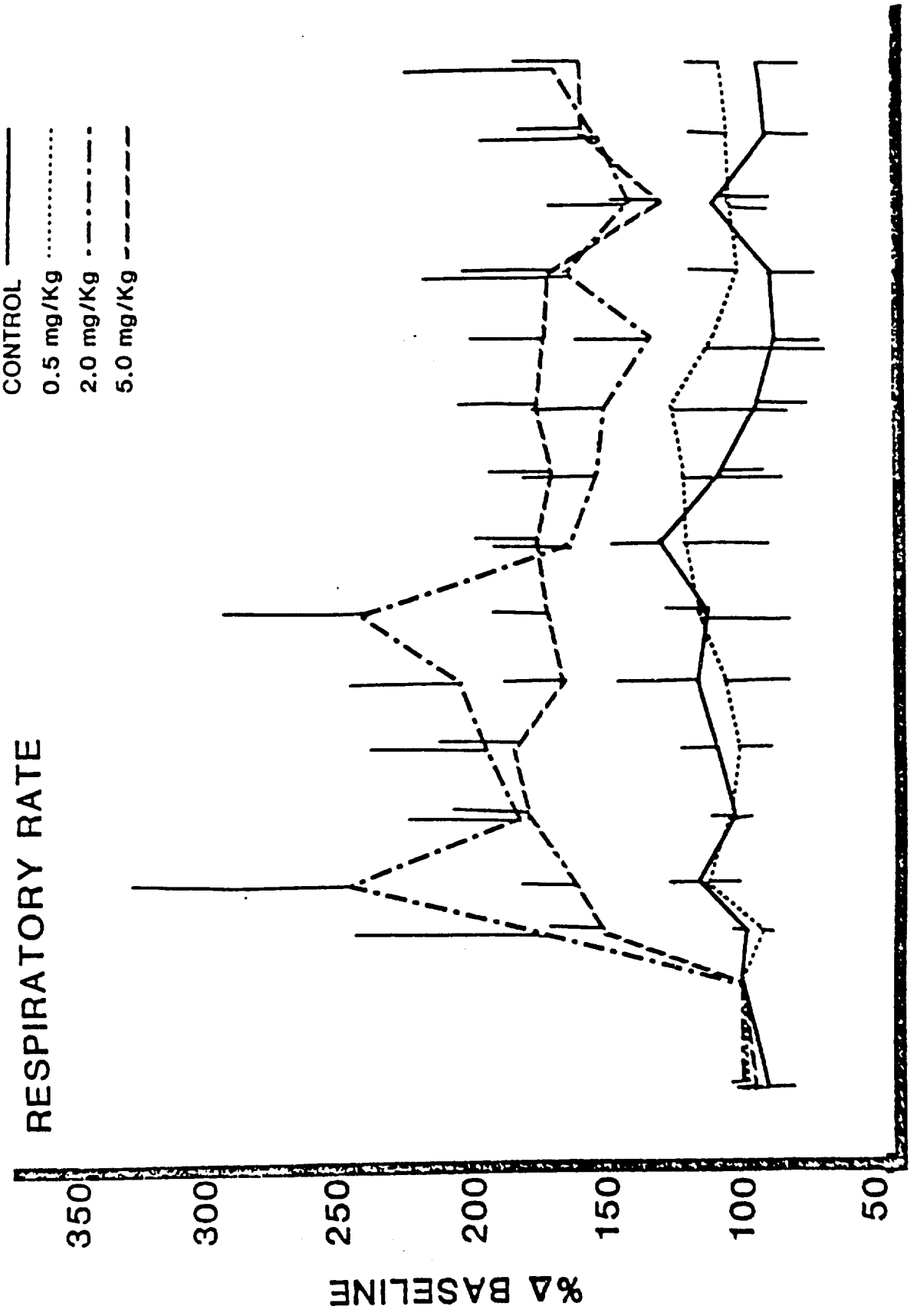

TIDAL VOLUME
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
140
120
100
80
60
-15
0
15
35
55
75
95
115
135
Infusion
minutes

figure 2


RESPIRATORY RATE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
350
300
250
200
150
100
50
-15
0
15
35
55
75
95
115
135
minutes
infusion

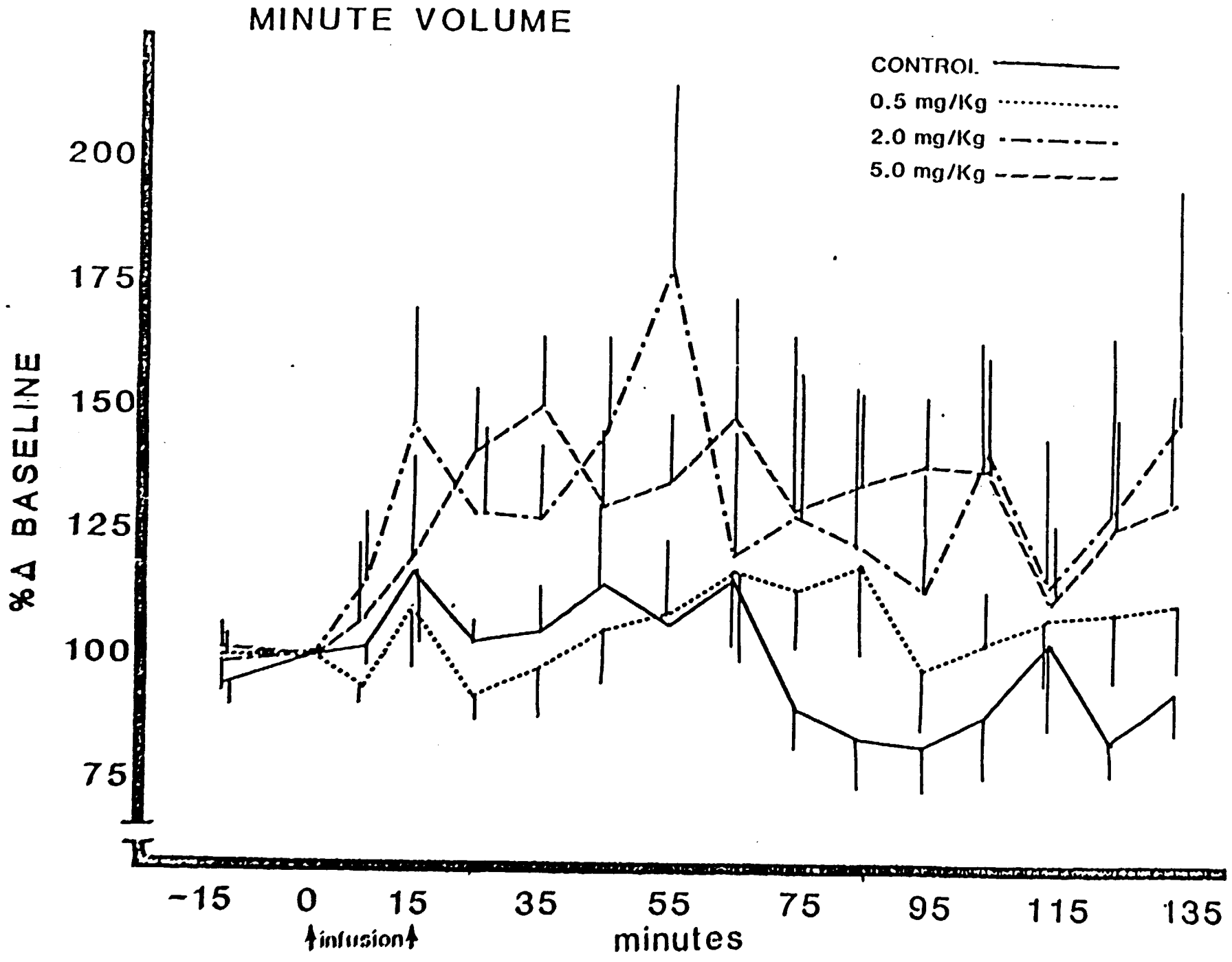

MINUTE VOLUME
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
200
175
150
125
100
75
%Δ BASELINE
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 5

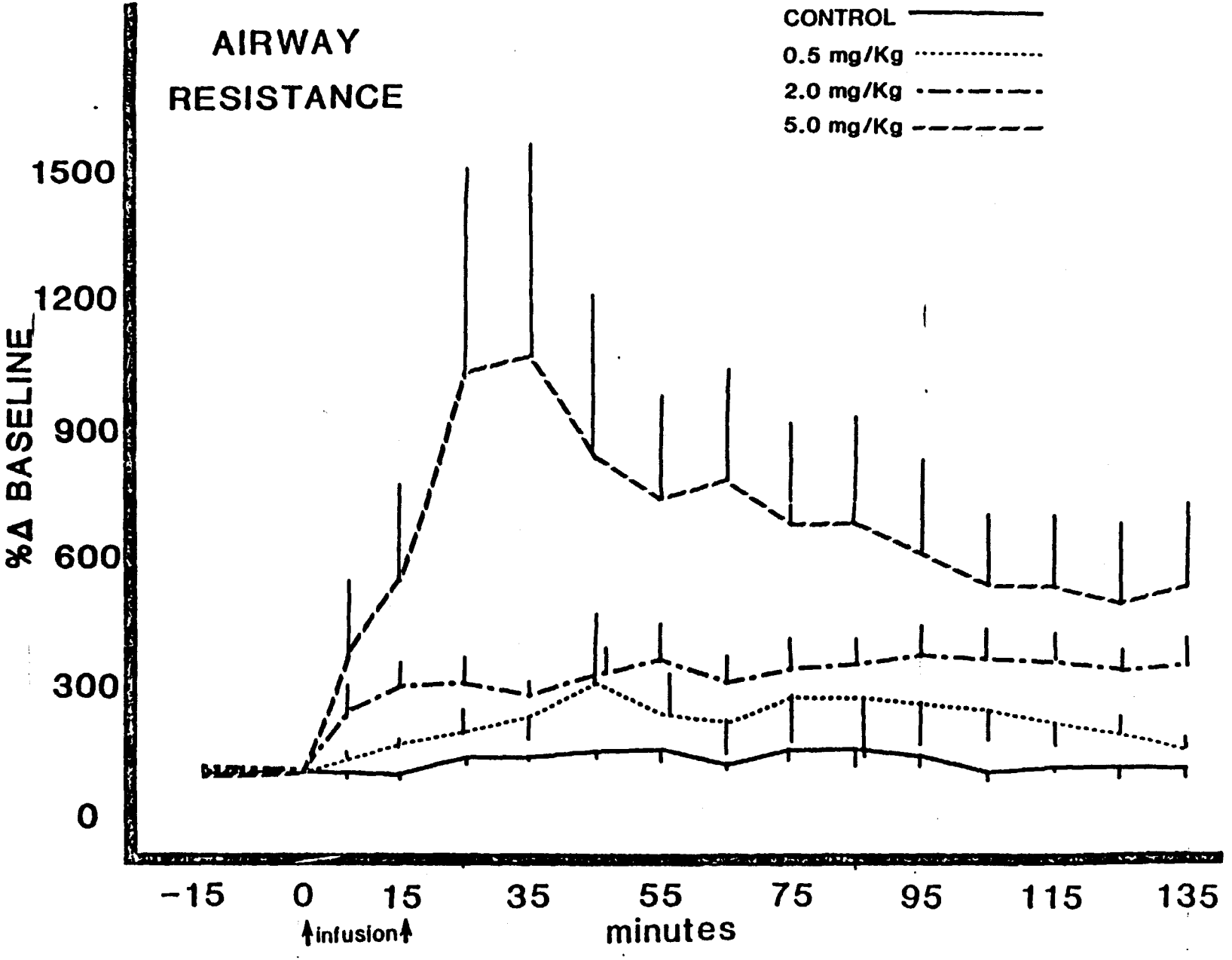

AIRWAY
RESISTANCE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
1500
1200
900
600
300
0
-15
0
15
35
55
75
95
115
135
infusion
minutes

figure 14

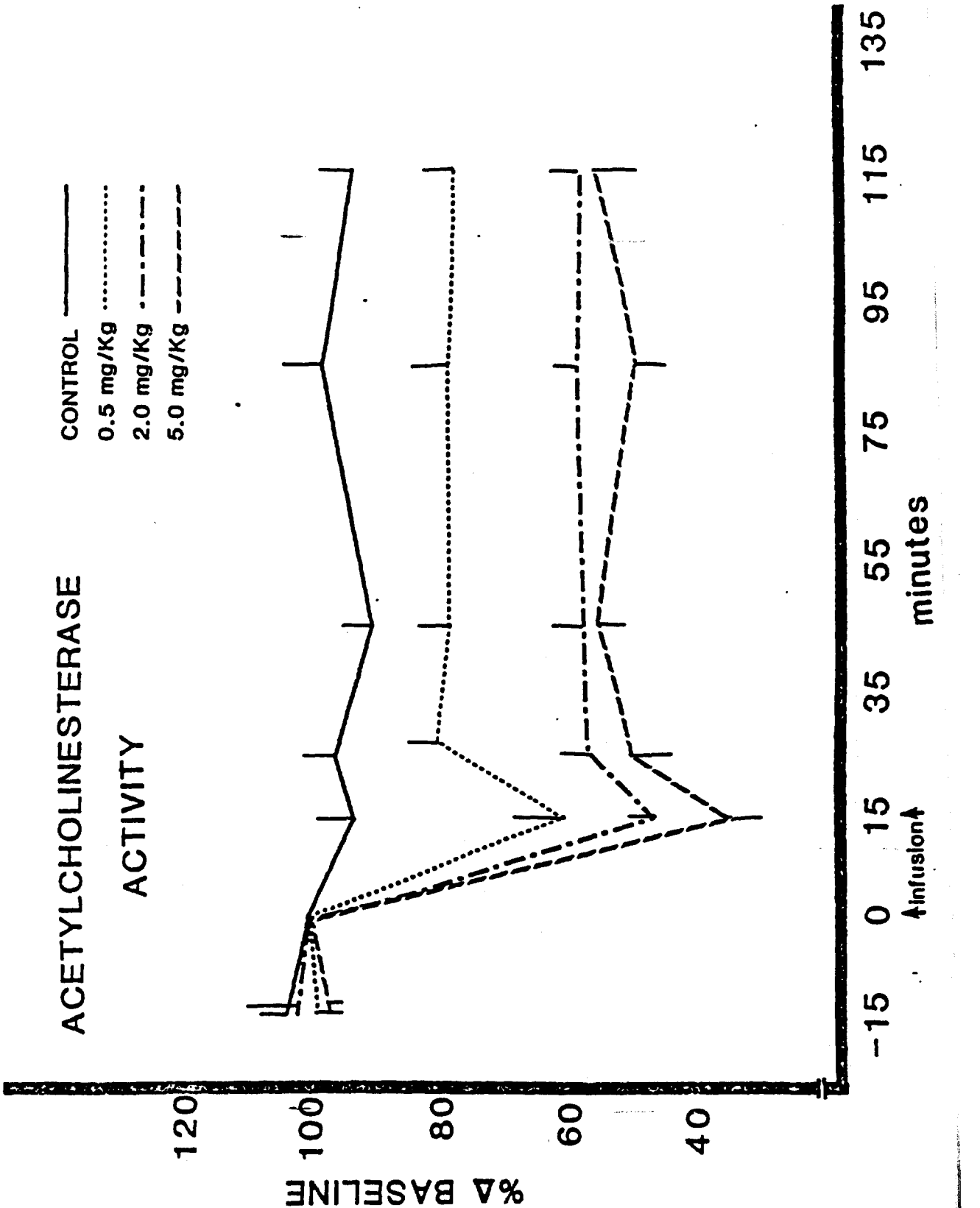

ACETYLCHOLINESTERASE
ACTIVITY
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
120
100
80
60
40
-15
0
15
35
55
75
95
115
135
minutes
Infusion

figure 8

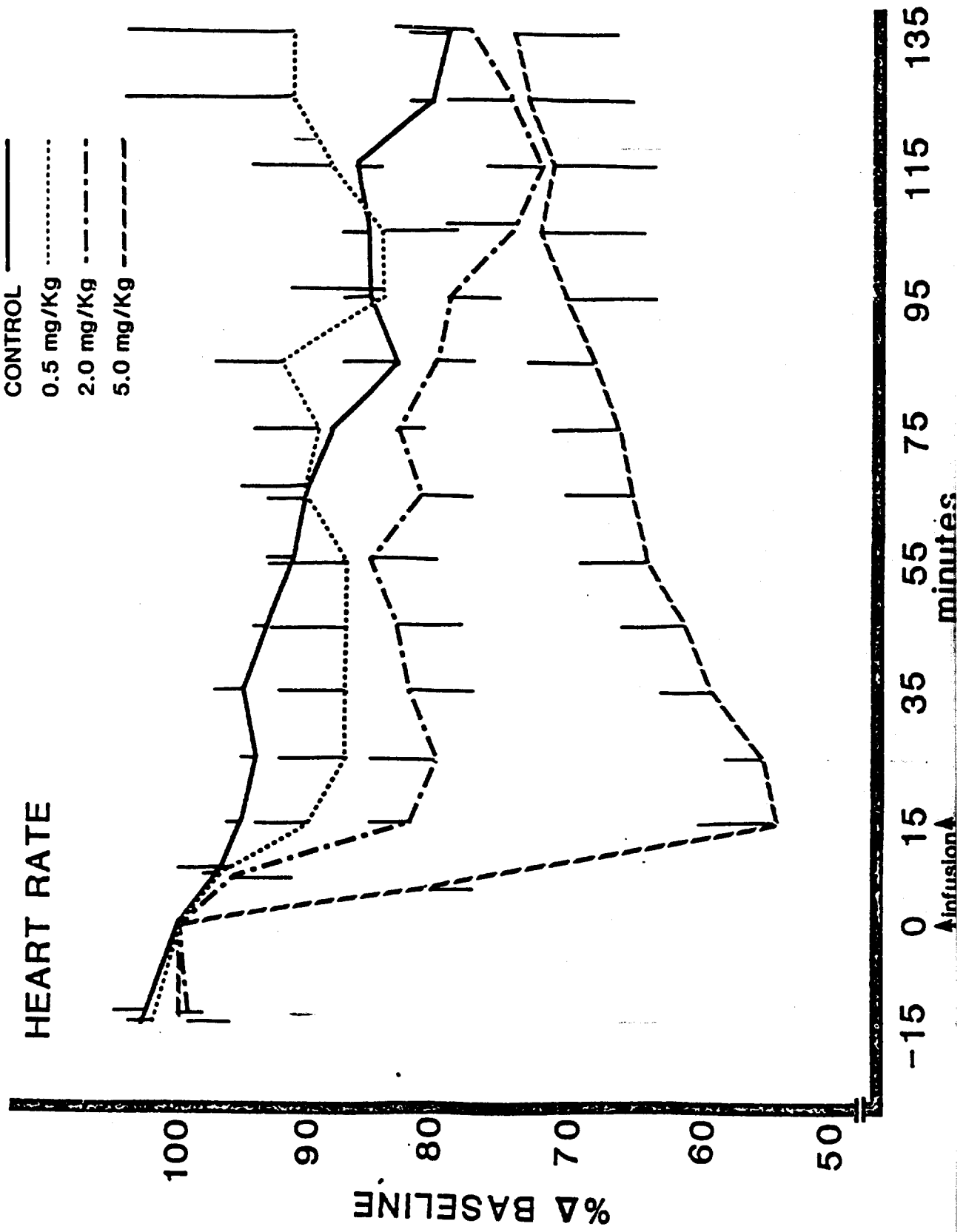

HEART RATE
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
100
90
80
70
60
50
-15
0
15
35
55
75
95
115
135
minutes
Δinfusion

figure 9

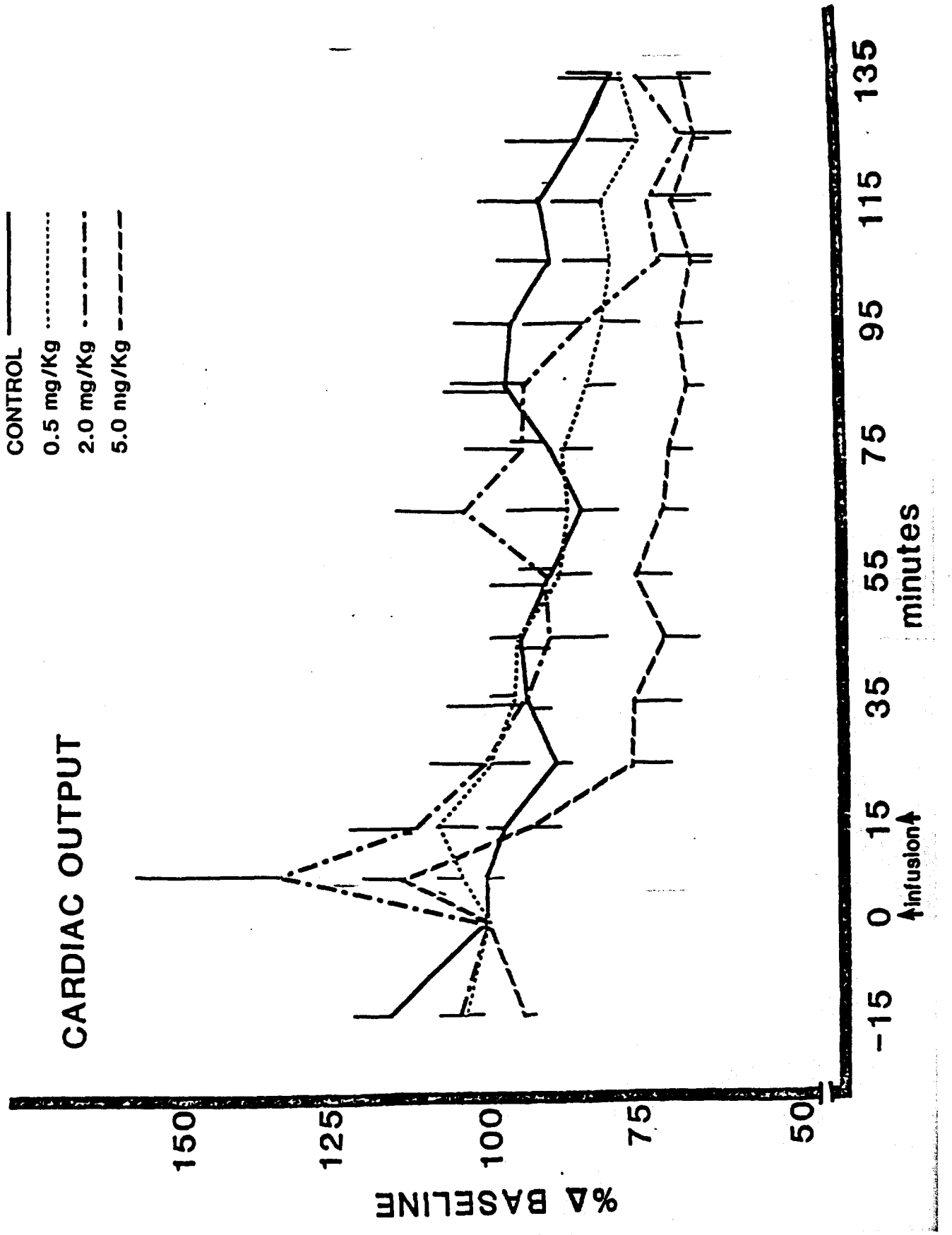

CARDIAC OUTPUT
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
150
125
100
75
50
-15
0
15
35
55
75
95
115
135
minutes
Infusion

Figure 9a

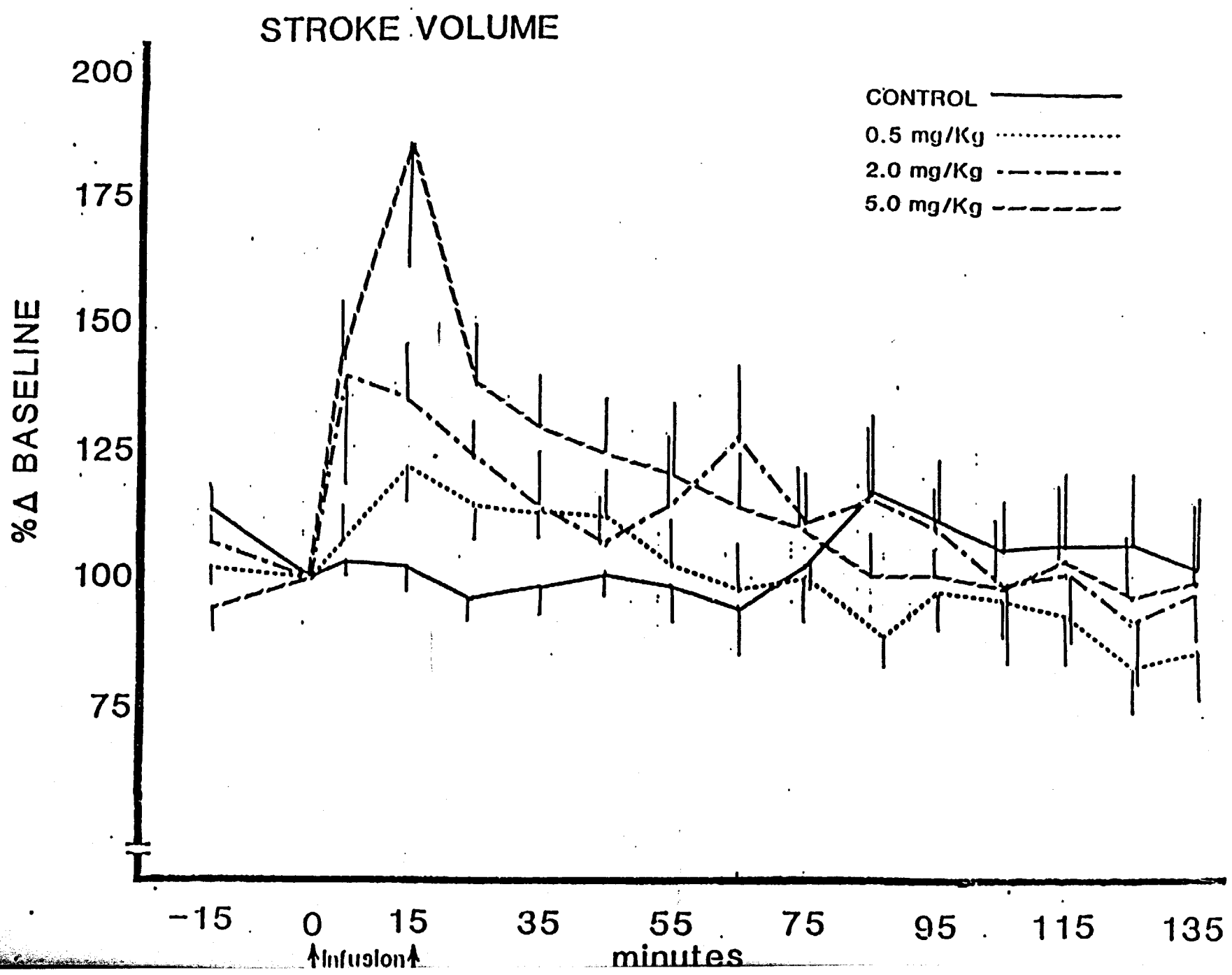

STROKE VOLUME
CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
%Δ BASELINE
200
175
150
125
100
75
−15
0
15
35
55
75
95
115
135
Infusion
minutes

figure 15

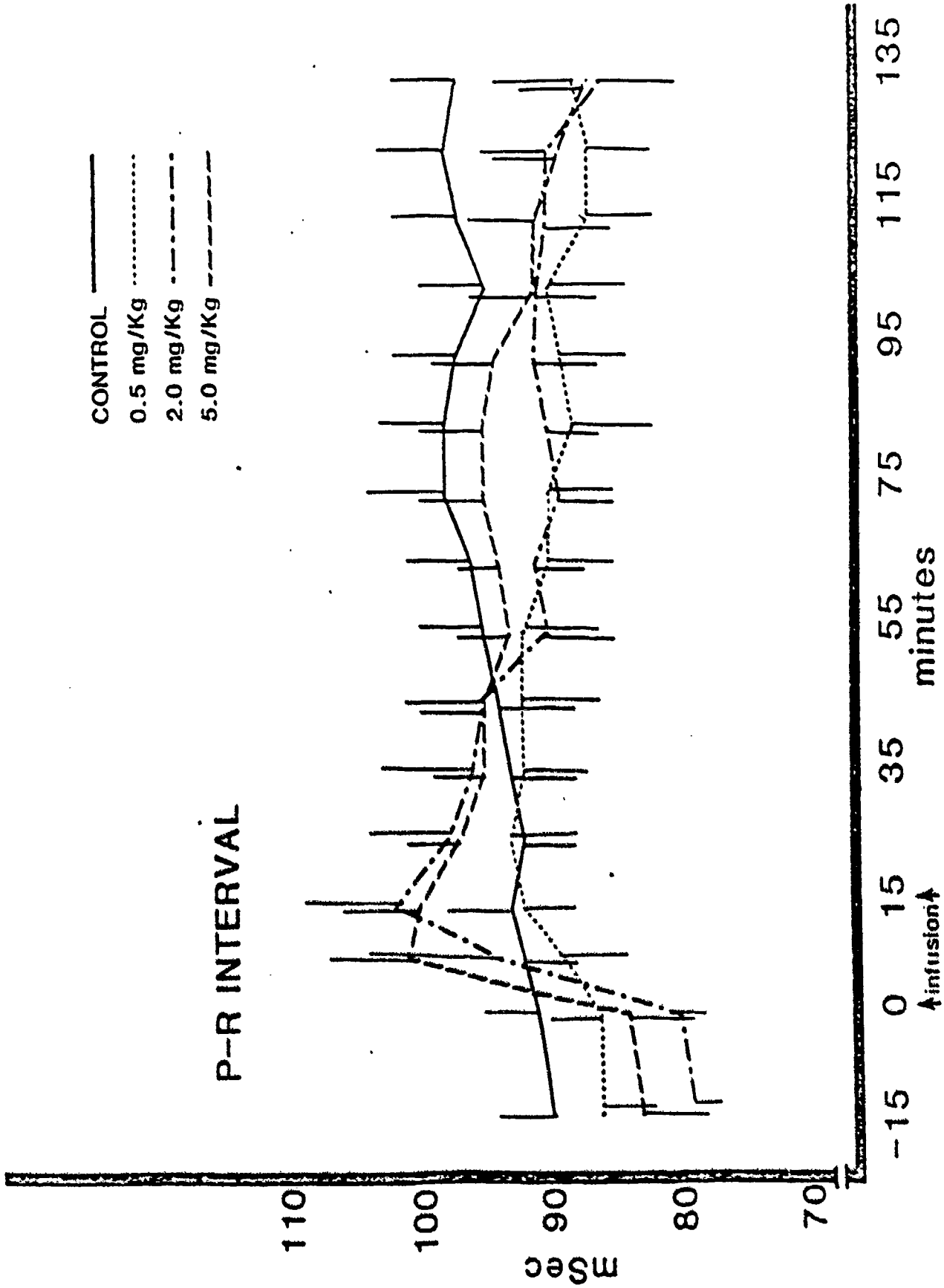
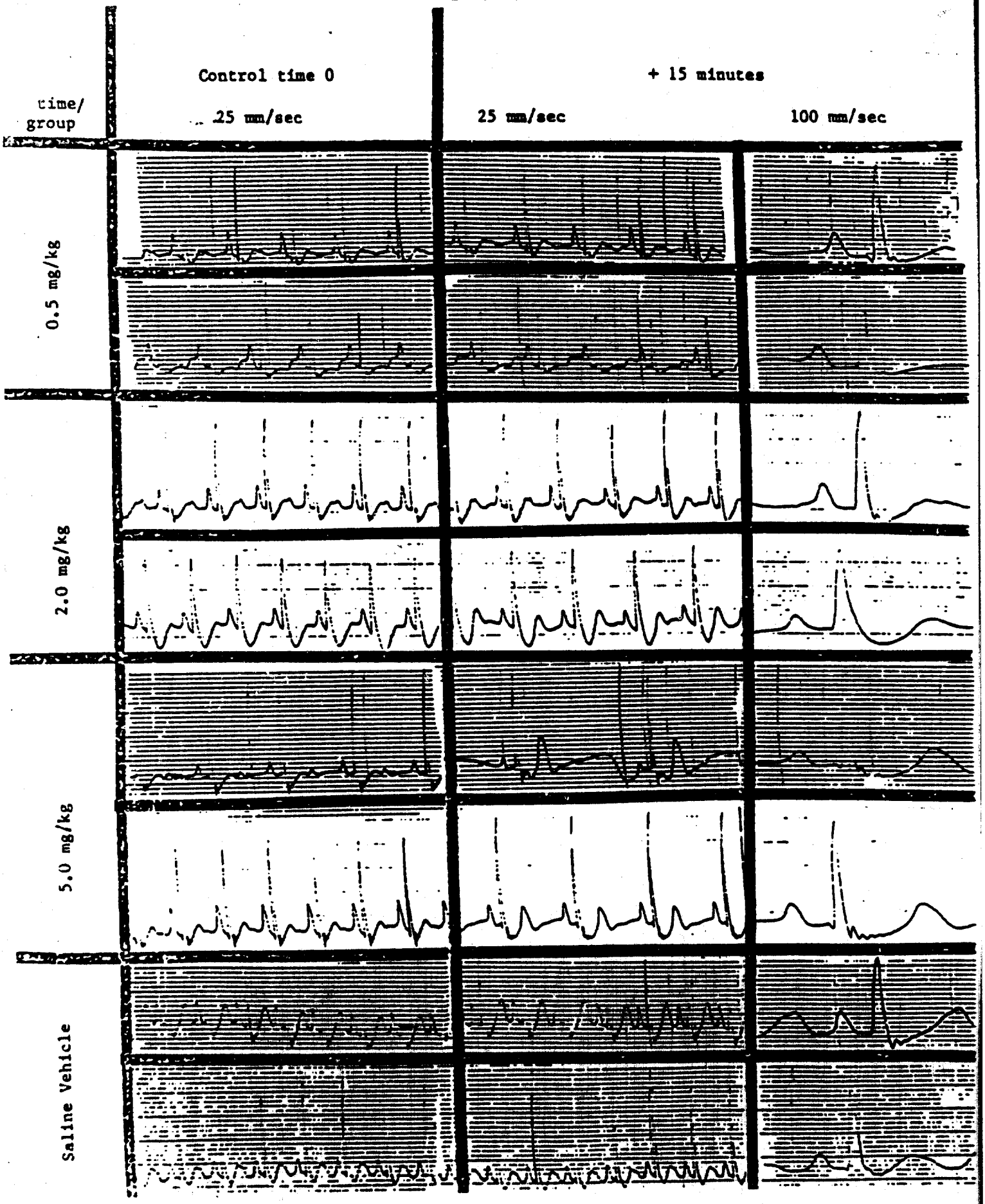

CONTROL
0.5 mg/Kg
2.0 mg/Kg
5.0 mg/Kg
P-R INTERVAL
mSec
110
100
90
80
70
-15
0
15
35
55
75
95
115
135
minutes
infusion

Figure 16

Representative ECG tracings

Lead II



Section II

PROTOCOL

I. INTRODUCTION

Chloroquine and mefloquine are both antimalarial drugs which have effects upon cardiac impulse formation and contractility (Arora and Lal, 1963; Hemwell and Di Palma, 1979; Caldwell and Nash, 1977). Use of mefloquine following chloroquine administration where resistance to chloroquine therapy might arise is a distinct clinical possibility. Since chloroquine has a slow rate of elimination after dosing [T-½ of 139-333 hr in man (Gustafsson et al., 1983], the strong possibility exists for combination effects. It is possible that the effects of these agents upon automaticity and contractility of the heart are more than additive, i.e. synergistic or potentiative.

II. PURPOSE

We will examine the ability of chloroquine and mefloquine separately, at a dose in the middle of their dose-response curves, and in combination, at half these doses, to alter the automaticity (initiation of impulses) and rhythmicity of both the atrium and ventricle, and pressure and contractility of the left ventricle. Classically, to determine if two agents having a qualitatively similar action interact to give a lesser or greater than additive effect, half of the ED_{50} (dose producing 50% of the maximum response) of each agent is given in combination and this total effect is compared with the sum of the responses to the ED_{50} of each agent given separately (Gaddum, 1959). For the purpose of determining dose-response relationships, preliminary experiments have been performed (see below).

III. PRELIMINARY EXPERIMENTS

Using the experimental preparation and procedure listed below (see IV and V), we have performed two experiments each with chloroquine or mefloquine to determine their actions on cardiac automaticity. Chloroquine or mefloquine were infused i.v. at doses of 2 mg/kg or 4 mg/kg, respectively, over 10 mins. Automaticity was assessed at four successive 10 min intervals. Cumulative effects of each of these drugs were assessed by repeating this process until 8 and 16 mg/kg of chloroquine or mefloquine had been given. Stable estimates of automaticity were obtained between 20 and 30 mins after drug administration. Both agents reduced automaticity of the ventricle in a linear dose-related manner with approximately half the maximum response occurring at 4 and 8 mg/kg of chloroquine and mefloquine, respectively.

IV. EXPERIMENTAL PREPARATION

Pure bred beagle dogs of either sex, 9 months and older, weighing between 9.0 and 14.0 kg will be purchased from Riglan Animal Care Systems. The dogs are guaranteed to be in excellent health and will be quarantined upon arrival at the University of Tennessee campus. Prior to their use, the University staff veterinarians will certify that they are in excellent health. The dogs will be anesthetized with pentobarbital Na, 30 mg/kg intravenously, and maintained with supplemental injections of pentobarbital Na, as necessary, to maintain stable anesthesia.

A femoral artery will be catheterized with polyethylene tubing filled with heparinized saline advanced to the thoracic aorta and connected to a Statham P23AC pressure transducer for measurement of arterial blood pressure.

One cephalic vein will be catheterized with polyethylene tubing, for infusion of drug. The contra-lateral cephalic vein will be catheterized in Group 3 (see below) when two drugs will be infused simultaneously. A Millar® Mikro-tip® pressure transducer catheter will be introduced through the left carotid artery; the tip will be positioned in the left ventricle for measurement of left ventricular pressure (LVP). The rising slope of the LVP will be differentiated to give dP/dt_{max}, an estimate of cardiac contractility.

A T-shaped cannula will be inserted into the trachea and the dog will breathe room air with the assistance of a Harvard respirator at 25 ml/kg tidal volume (10-15 breaths/minute).

The chest will be opened via a mid-sternal incision and the pericardium removed. Two plexiglass plates (1.5 x 0.5 cm) each having four platinum electrodes will be sewn to the surface of the left ventricle and right atrial appendage to produce good contact. Two electrodes from each plate will be used to record the electrogram. The remaining two electrodes on the ventricle and the atrium will be attached to a Grass Model SD9 stimulator and a Grass Model S4 stimulator respectively for electrical drive.

These studies will be conducted in a manner that adheres to the "Guide for the Care and Use of Laboratory Animals", Institute for Laboratory Animal Research, National Research Council, NIH Publication 85-23, revised 1985.

Drug Preparation WRAIR will supply mefloquine with assay report, we will purchase chloroquine with assay report from Sigma Chemical Company.

Both of the drugs involved in this study are water soluble to some degree and will be dissolved and administered in dextrose 5% in water (D5W) at 21°C. The D5W (inj., USP) will be purchased from Abbott laboratories. Mefloquine

HCl exhibits the lower solubility but can be dissolved to 2 mg/ml. A Cole-Palmer variable speed perisaltic infusion pump will be used to deliver a volume rate of 4 ml/kg of mefloquine HCl solution over 10 minutes. Chloroquine phosphate dissolves readily in water. A 4 mg/kg dose of chloroquine will be dissolved in 4 ml/kg of D5W and given over 10 minutes by the pump. For combination administration, chloroquine (2 mg/kg) and mefloquine HCl (4mg/kg) will each be dissolved in 2 ml/kg of D5W and simultaneously delivered over 10 min.

Conduction Blockade

Complete heart block by the method of Steiner and Kovalik (1968), will be achieved by injecting 0.1 ml of 40% formaldehyde into the atrial septum at the level of the A-V node and the adjoining common bundle of His. Injection will be accomplished via a 25 gage needle placed at a depth of 0.5 to 1.0 cm below the groove between atrium and aorta. Complete heart block is verified by lead II EKG recording.

V. EXPERIMENTAL PROCEDURE

Our procedure is patterned after those described by Afonso et al. (1972) and Korte and Nash (1978). The dog will be allowed to equilibrate for 30 minutes following surgery and A-V blockade. Baseline measurements of the following variables will be taken: heart tissue rates-intrinsic atrial and ventricular rates, aortic blood pressure, left ventricular blood pressure (LVP), and LV dP/dt_{max}.

The atria and ventricles will then be simultaneously overdriven for 2 minutes with a square wave D.C. pulse of 5 mSec duration at a current strength

3 times driving threshold (Korte and Nash, 1978). Current threshold will be determined by the minimum current required to drive the atria and ventricles. We will drive the atria at 200 beats/min and the ventricle at 150 beats/min. Immediately following the period of overdrive, assessment of automaticity will be made as follows:

A. Asystole period from the cessation of stimulation until first depolarization.

B. The number of depolarizations in the 30 seconds immediately following cessation of stimulation.

C. The time required for the first ten depolarizations following cessation of stimulation.

Observation Periods

Measurements of cardiovascular function and automaticity as stated above will be made 15 min before, just prior and at 10 minute intervals for the 90 minutes following the drug infusion.

Analysis of Data

The responses of all variables for each time point for each group will be tabulated, averaged and an estimate of variability (S.E.M.) will be calculated. Differences in responses among the groups will be determined by analysis of variance.

Experimental Groups - Each group will be comprised of six dogs

1. Mefloquine (8mg/kg base) infused over 10 minutes. Wait 10 minutes for drug distribution and then proceed with experiment.

2. Chloroquine (4 mg/kg base) infused over 10 minutes. Wait 10 minutes then proceed with experiment.

3. Mefloquine (4 mg/kg) and Chloroquine (2 mg/kg) infused simultaneously in separate veins over 10 minutes. Wait 10 minutes then proceed with experiment.
4. Vehicle control infusion of D5W (5 ml/kg) over 10 min. Wait 10 minutes and proceed with experiment.

The conduct of these studies shall comply with the GOOD LABORATORY PRACTICES (GLP) regulations as published in the Federal Register, Volume 43 (247), 22 December 1978, Part II, pp 59, 986-60,020 (and all subsequent addenda) as per Task Order. The contracter shall notify the contracting officer (301) 663-2987 and the COTR (302) 427-5148 by telephone immediately upon announcement by a representative of the FDA of an inspection of studies performed under this contract. In addition to the FDA representative, the USAMRDC-appointed COTR shall have access to the contractor's records. With reference to paragraph 58,195(g) of the GLP regulations, the contractor shall notify the contracting officer in writing, in addition to the FDA, should the contractor go out of business and/or transfer the records during the periods prescribed in paragraph 58,195. On expiration or termination of the contract, the contractor shall notify the contracting officer of any remaining unused test articles.

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Quality Assurance Officer

Clinton B. Nash, Ph.D.

M.A. Chryssanthis, B.S.

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